(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 May 2008 (22.05.2008)

PCT

(10) International Publication Number
WO 2008/060332 A2

(51) International Patent Classification:
A61K 31/4709 (2006.01)

(21) International Application Number:
PCT/US2007/012936

(22) International Filing Date: 1 June 2007 (01.06.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/810,748 2 June 2006 (02.06.2006) US
PCT/US2006/32405
17 August 2006 (17.08.2006) US
60/904,348 28 February 2007 (28.02.2007) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

WO 2008/060332 A2

(54) Title: METHODS FOR TREATING OR REDUCING MUSCLE FATIGUE

(57) Abstract: The invention provides methods for treating muscle fatigue with compounds of the invention. The invention relates to compositions and methods for treating, preventing or reducing muscle fatigue by administering compounds that stabilize ryanodine receptors (RyR), which regulate calcium channel functioning in cells. The invention provides methods to treat muscle fatigue in a subject suffereing from a wide variety of acute and chronic pathologies, neurological or genetic diseases or conditions, including but not limited to cardiac disease, HIV infection and IDS, muscular dystrophy, cancer, malnutrition, exercise- induced muscle fatigue, age-associated muscle fatigue, renal disease and renal failure. The invention provides methods for treating a subject suffering from muscle fatigue as a result of sustained, prolonged and/or strenuous exercise, or chronic stress.

METHODS FOR TREATING OR REDUCING MUSCLE FATIGUE

[0001] This application claims the benefit of priority of International Application PCT/US2006/32405 filed August 17, 2006; this application also claims the benefit of priority of U.S. Application Nos: 60/810,748 filed June 2, 2006, and 60/904,348 filed February 28, 2007. The disclosures of these applications in their entirety are hereby incorporated by reference into this application.

[0002] This invention was made with government support under DoD DARPA grant No. W911NF-05-1-04. As such, the United States government has certain rights in this invention.

[0003] Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

FIELD OF THE INVENTION

[0004] This invention is in the field of skeletal muscle fatigue, muscle conditions and disorders, and treatments thereof.

BACKGROUND

[0005] The sarcoplasmic reticulum (SR) functions, among other things, as a specialized intracellular calcium (Ca^{2+}) store. Channels in the SR called ryanodine receptors (RyRs) open and close to regulate the release of Ca^{2+} from the SR into the intracellular cytoplasm of the cell. Release of Ca^{2+} into the cytoplasm from the SR increases cytoplasmic Ca^{2+} concentration. Open probability (Po) of the RyR receptor refers to the likelihood that the RyR channel is open at any given moment, and therefore capable of releasing Ca^{2+} into the cytoplasm from the SR.

[0006] There are three types of ryanodine receptors, all of which are Ca^{2+} channels: RyR1, RyR2, and RyR3. RyR1 is found predominantly in skeletal muscle as well as other tissues, RyR2 is found predominantly in the heart as well as other tissues, and RyR3 is found in the brain as well as other tissues. The RyR channels are formed by four RyR polypeptides in association with four FK506 binding proteins (FKBPs), specifically FKBP12 (calstabin1) and FKBP12.6 (calstabin2). Calstabin1 binds to RyR1, calstabin2 binds to RyR2, and calstabin1

binds to RyR3. The FKBP proteins (calstabin1 and calstabin2) bind to the RyR channel (one molecule per RyR subunit), stabilize RyR-channel functioning, and facilitate coupled gating between neighboring RyR channels, thereby preventing abnormal activation of the channel during the channel's closed state.

[0007] Protein kinase A (PKA) binds to the cytoplasmic surface of the RyR receptors. PKA phosphorylation of the RyR receptors causes partial dissociation of calstabins from RyRs. Dissociation of calstabin from RyR causes increased open probability of RyR, and therefore increased Ca^{2+} release from the SR into the intracellular cytoplasm.

[0008] Ca^{2+} release from the SR in skeletal muscle cells and heart cells is a key physiological mechanism that controls muscle performance, because increased concentration of Ca^{2+} in the intracellular cytoplasm causes contraction of the muscle. Excitation-contraction (EC) coupling in skeletal muscles involves electrical depolarization of the plasma membrane in the transverse tubule (T-tubule), which activates voltage-gated L-type Ca^{2+} channels (LTCCs). LTCCs trigger Ca^{2+} release from the SR through physical interaction with RyR1. The resulting increase in cytoplasmic Ca^{2+} concentration induces actin-myosin interaction and muscle contraction. To enable relaxation, intracellular Ca^{2+} is pumped back into the SR via SR Ca2+-ATPase pumps (SERCAs), which is regulated by phospholamban (PLB) depending on the muscle fiber type.

[0009] Fatigue is the process whereby skeletal muscles become weaker with repeated or intense use such as exercise, or as a result of an illness, disorder or disease. Fatigue can result in task failure and it can be a pronounced symptom in a variety of medical conditions including heart failure, renal failure, cancer, and various muscular dystrophies. Over the past decade, it has become evident that the two dominant classical explanations of muscle fatigue, namely, accumulation of lactic acid and intracellular acidosis, do not cause fatigue. In fact, both may be protective mechanisms during high intensity exercise to prevent fatigue (Allen and Westerblad 2004; Pedersen, Nielsen et al. 2004)

[0010] Muscle contraction depends on the efficient coupling of electrical stimulation of the muscle surface to Ca^{2+} release via the ryanodine receptor, the SR Ca^{2+} release channel, to the generation of actinmyosin cross bridges. It is evident, then, that a defect in excitation-contraction coupling that resulted in a reduction in the amplitude of the Ca^{2+} transient would, among other effects, result in impaired contraction and force generation through ineffective

myosin cross bridge formation. Eberstein and Sandow suggested inhibition of Ca^{2+} release as a likely factor in the fatigue process (Eberstein and Sandow 1963). Reductions in the amplitude of Ca^{2+} release evoked during fatiguing stimulation have been reported in multiple muscle preparations(Allen, Lee et al. 1989; Westerblad and Allen 1991; Allen and Westerblad 2001). The time course of recovery from fatigue parallels the time course over which prolonged depression of Ca^{2+} release is observed (Westerblad, Bruton et al. 2000).

[0011] SR Ca^{2+} leak was documented in myofibers following intense exercise and in a model of muscular dystrophy (Wang, Weisleder et al. 2005), possibly due to defective skeletal ryanodine receptors (RyR1s). Chronic activation of the sympathetic nervous system (SNS) in the context of heart failure promotes intrinsic skeletal muscle fatigue due to depletion of the phosphodiesterease PDE4D3 from the RyR1 complex, RyR1 PKA hyperphosphorylation at Serine 2844, calstabin1 depletion from the RyR1 complex, and a gain-of-function channel defect (Reiken, Lacampagne et al. 2003). RyR1 dysfunction in the skeletal muscle leads to altered local subcellular Ca^{2+} release events and impaired global calcium transients (Ward, Reiken et al. 2003). JTV519, (4-[3-(4-benzylpiperidin-1-yl)propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride- a 1,4-benzothiazepine has been shown to be a modulator of RyR calcium-ion channels), given in the context of a murine model of heart failure, was able to improve skeletal muscle function, as assessed by an ex vivo isolated muscle fatiguing protocol, five weeks after left coronary artery ligation. JTV519's beneficial effect on muscle fatigue was not solely due to cardiac improvement, as a beneficial effect was still seen when the drug was given to calstabin2 deficient mice which derive no cardiac benefit from treatment with JTV519. Thus, it has been postulated that JTV519 directly affects muscle function (Wehrens, Lehnart et al. 2005). In the context of chronic exercise, identical changes in the RyR1 macromolecular complex, namely depletion of PDE4D3 from the RyR1 complex, RyR1 PKA hyperphosphorylation at Serine 2844, and calstabin1 depletion from the RyR1 complex are related in a time-dependent and activity-dependent manner with repeated intense exercise in a mouse model. These biochemical changes in the RyR1 macromolecular complex regulation and function are stable following prolonged exercise and recover slowly over days to weeks. It has therefore been proposed that RyR1 Ca^{2+} leak limits peak muscle performance and mediates muscle damage during prolonged, stressful exercise.

[0012] The contraction of striated muscle is initiated when calcium (Ca^{2+}) is released from tubules within the muscle cell known as the sarcoplasmic reticulum (SR). Calcium release channels, called ryanodine receptors (RyR), on the SR are required for excitation-contraction (EC) coupling. The type 2 ryanodine receptor (RyR2) is found in the heart, while the type 1 ryanodine receptor (RyR1) is found in skeletal muscle. The RyR1 receptor is a tetramer comprised of four 565,000 dalton RyR1 polypeptides and four 12,000 dalton FK-506 binding proteins (FKBP12). FKBP12s are regulatory subunits that stabilize RyR channel function (Brillantes et al., 1994) and facilitate coupled gating between neighboring RyR channels (Marx et al., 1998); the latter are packed into dense arrays in specialized SR regions that release intracellular stores of Ca^{2+}, thereby triggering muscle contraction. In addition to FKBP12, the RyR1 macromolecular complex also includes the catalytic and regulatory subunits of PKA, and the phosphatase PP1 (Marx et al., 2001).

[0013] One FKBP12 molecule is bound to each RyR1 subunit. Dissociation of FKBP12 significantly alters the biophysical properties of the channels, resulting in the appearance of subconductance states, and increased open probability (P_o) of the channels (Brillantes et al., 1994; Gaburjakova et al., 2001). In addition, dissociation of FKBP12 from RyR1 channels inhibits coupled gating resulting in channels that gate stochastically rather than as an ensemble (Marx et al., 1998). Coupled gating of arrays of RyR channels is thought to be important for efficient EC coupling that regulates muscle contraction (Marx et al., 1998). FKBPs are cis-trans peptidyl-prolyl isomerases that are widely expressed and subserve a variety of cellular functions (Marks, 1996). FKBP12s are tightly bound to and regulate the function of the skeletal (RyR1) (Brillantes et al., 1994; Jayaraman et al., 1992) and cardiac (RyR2) (Kaftan et al., 1996) muscle Ca^{2+} release channels, as well as a related intracellular Ca^{2+} release channel known as the type 1 inositol 1,4,5-triphosphate receptor (IP3R1) (Cameron et al., 1997), and the type I transforming growth factor .beta. (TGF.beta.) receptor (T.beta.RI) (Chen et al., 1997).

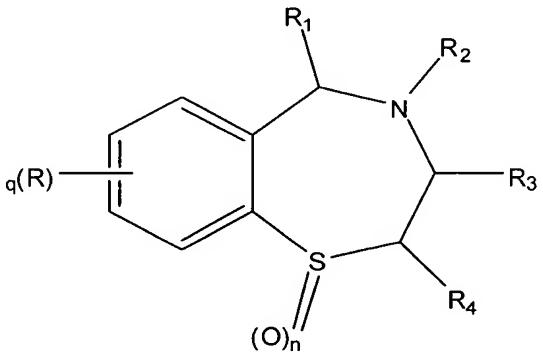
[0014] Co-pending application U.S. Serial No. 10/794,218, the contents of which are hereby incorporated by reference, discloses methods of treating defective skeletal muscle function during heart failure by administering an agent which inhibits dissociation of FKB12 binding protein from RyR1 receptor.

[0015] Co-pending U.S. Patent Applications No. 11/212,309 and U.S. Patent Application No. 11/212,413, the contents of which are hereby incorporated by reference, disclose methods of using novel benzothiazepine derivatives to treat and prevent disorders and diseases associated with the RyR receptors, including skeletal muscular disorders and diseases such as skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence.

[0016] There is a need to identify new agents effective for treating or preventing muscle fatigue that is stress or exercise induced or that results from diseases associated with the RyR receptors that regulate calcium channel functioning in cells, including cardiac disease or disorder, defective skeletal muscle function, HIV Infection, AIDS, muscular dystrophy, cancer, malnutrition, exercise-induced muscle fatigue, age-associated muscle fatigue, renal disease, and renal failure.

SUMMARY OF THE INVENTION

[0017] In one aspect, the invention provides a method for treating, preventing or ameliorating muscle conditions, diseases or disorders, the method comprising administering to the subject a therapeutically effective amount of a compound of Formula-I. In certain aspects, the invention provides a method of treating, preventing, or ameliorating of muscle fatigue in a subject, comprising administering to the subject a therapeutically effective amount of a compound having the Formula-I:



wherein,

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, -NH_2, -NO_2, -CN, -CF_3, -OCF_3, -N_3, -SO_3H, -$S(=O)_2$alkyl, -S(=O)alkyl, -$OS(=O)_2CF_3$, acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted or unsubstituted;

R_1 is selected from the group consisting of H, oxo, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be substituted or unsubstituted;

R_2 is selected from the group consisting of H, $-C(=O)R_5$, $-C(=S)R_6$, -SO_2R_7, $-P(=O)R_8R_9$, -$(CH_2)_m$-R_{10}, alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be substituted or unsubstituted;

R_3 is selected from the group consisting of H , -CO_2Y, -C(=O)NHY, acyl, -O-acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be substituted or unsubstituted; and wherein Y is selected from the group consisting of H, alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl, and wherein each alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be substituted or unsubstituted;

R_4 is selected from the group consisting of H, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be substituted or unsubstituted;

R_5 is selected from the group consisting of $-NR_{15}R_{16}$, -$(CH_2)_z$$NR_{15}R_{16}$, -$NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, -C(=O)$NHNR_{15}R_{16}$, -CO_2R_{15}, -C(=O)$NR_{15}R_{16}$, -CH_2X, acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted, and wherein z is 1, 2, 3, 4, 5, or 6;

R_6 is selected from the group consisting of $-OR_{15}$, $-NHNR_{15}R_{16}$, -NHOH, $-NR_{15}R_{16}$, $-CH_2X$, acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_7 is selected from the group consisting of $-OR_{15}$, $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-CH_2X$, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_8 and R_9 independently are selected from the group consisting of OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_{10} is selected from the group consisting of $-NR_{15}R_{16}$, OH, $-SO_2R_{11}$, $-NHSO_2R_{11}$, $C(=O)(R_{12})$, $NHC=O(R_{12})$, $-OC=O(R_{12})$, and $-P(=O)R_{13}R_{14}$;

R_{11}, R_{12}, R_{13}, and R_{14} independently are selected from the group consisting of H, OH, NH_2, $-NHNH_2$, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

X is selected from the group consisting of halogen, $-CN$, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-NR_{15}R_{16}$, $-OR_{15}$, $-SO_2R_7$, and $-P(=O)R_8R_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH_2, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted; and

optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted;

the nitrogen in the benzothiazepine ring may optionally be a quaternary nitrogen; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof. In certain embodiments, the compound is not S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, or S100. In another embodiment, the compound is not S4, S7, S20, S24, S25, S26, S27, or S36. In one embodiment, the compound is not JTV-519.

[0018] In certain embodiments, the methods treat symptoms. In other embodiments, the methods treat cause and/or symptoms.

[0019] In certain embodiments, the compound is described by a chemical formula selected from the group consisting of Formula I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p. In other embodiments, the compound is described by a chemical formula selected from the group consisting of Formula I-a, I-b, I-e, I-f, I-g, I-h, I-l, I-n, I-o, and I-p. In other embodiments, the compound is described by a chemical formula selected from the group consisting of Formula I-n, I-o, and I-p. In other embodiments, the compound is selected from the group consisting of S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99,S100, S101, S102, S103, S104, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123. In other embodiments, the compound is selected from the group consisting of S47, S48, S50, S51, S59, S64, S74, S75, S77, S85, S101, S102, S103, S107, S109, S110, S111, S117, and S121. In other embodiments, the compound is selected from the group consisting of S68, S101, S102, S103, S107, S109, S110, S111, S117, and S121. In one embodiment, the compound is S107.

[0020] In other embodiments of the methods, muscle fatigue is due a skeletal muscle disease or disorder. In other embodiments, the skeletal muscle disease or disorder is associated with abnormal function of an RyR1 receptor. In certain embodiments, the methods treat muscle fatigue due to myopathy. In other embodiments, the methods treat muscle fatigue due to muscluar dystrophy. In other embodiments, the methods treat muscle fatigue due to central core disease. In other embodiments, the methods treat malignanat hyperthermia. In other embodiments, the muscle fatigue is exercise-induced muscle fatigue, or age-related muscle fatigue. In other embodiments, the exercise-induced muscle fatigue is due to prolonged exercise or high-intensity exercise.

[0021] In other embodiments, the subject is suffering from neuropathy, a neurological disease or disorder, a seizure condition, a genetic disease or disorder, a cardiac disease or disorder, an infectious disease, HIV infection, AIDS, a myopathy, a muscular dystrophy, cancer, malnutrition, renal disease, or renal failure. In certain embodiments, the cardiac disease or disorder is selected from the group consisting of an irregular heartbeat condition, an exercise-induced irregular heartbeat, congestive heart failure, chronic obstructive pulmonary disease, high blood pressure or any combination thereof. In other embodiments, the irregular heartbeat condition is selected from the group consisting of atrial or ventricular arrhythmia, atrial or ventricular fibrillation; atrial or ventricular tachyarrhythmia; atrial or ventricular tachycardia, catecholaminergic polymorphic ventricular tachycardia (CPTV), and exercise-induced variants thereof.

[0022] In other embodiments, the myopathy is a congenital myopathy. In other embodiments, the myopathy is selected from the group consisting of muscular dystrophies, central core diseases, myopathy with cores and rods, mitochondrial myopathies, endocrine myopathies, glycogen storage diseases, myoglobinurias, dermatomyositis, myositis ossificans, familial periodic paralysis, polymyositis, inclusion body myositis, neuromyotonia, and stiff-man syndrome. In other embodiments, the myopathy is a muscular dystrophy, central core disease, or malignant hyperthermia. In other embodiments, the muscular dystrophy is selected from the group consisting of Duchenne muscular dystrophy, facioscapulohumeral dystrophy, limb girdle muscular dystrophy, myotonic muscular dystrophy, myotonic dystrophy type I, myotonic muscular type II, Becker's muscular dystrophy, congenital muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy , myotonic muscular dystrophy, and

oculopharyngeal muscular dystrophy. In other embodiments, the glycogen storage disease is selected from the group consisting of Pompe's disease, Andersen's disease, and Cori's diseases. In other embodiments, the myoglobinuria is selected from the group consisting of McArdle's disease, Tarui disease, and DiMauro disease. In other embodiments, the mitochondrial myopathy is selected from the group consisting of Kearns-Sayre syndrome, MELAS syndrome, and MERRF syndrome. In other embodiments, is a non-human animal selected from the group consisting of a canine, an equine, a feline, a porcine, a murine, a bovine, an avian and an ovine animal. In one embodiment, the subject is a human.

[0023] In another aspect, the invention provides a method of treating, preventing, or ameliorating of muscle fatigue in a subject, comprising administering to the subject a therapeutically effective amount of a compound described by a chemical formula selected from the group consisting of S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99,S100, S101, S102, S103, S104, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123,or any enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites and prodrugs thereof. In certain embodiments, the compound is not S4, S7, S20, S24, S25, S26, S27, or S36. In one embodiment, the muscle fatigue is exercise-induced muscle fatigue. In another embodiment, the muscle fatigue is associated with any other disease or condition which is characterized with muscle fatigue. In one embodiment, the muscle fatigue is due to a myopathy. In another embodiment, the muscle fatigue is due to muscular dystrophy. In another embodiment, the muscle fatigue is due to central core disease. In certain embodiments, the copound is selected from the group consisting of S47, S48, S50, S51, S59, S64, S74, S75, S77, S85, S101, S102, S103, S107, S109, S110, S111, S117, and S121, or any enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof. In certain embodiments, the compound is selected from the group consisting of S101, S102, S103, S107, S109, S110, S111, S117, and S121, or any enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof. In one embodiment the compound is S107.

[0024] In certain embodiments, the subject is suffering from an exercise induced muscle fatigue, age-related muscle fatigue, myopathy, neurological disorder or neuropathy, an infectious disease, a chronic disorder, a genetic disease, or any other disease or disorder associated with muscle fatigue. In certain embodiments, the subject is suffering from a cardiac disease or disorder, HIV Infection, AIDS, muscular dystrophy, cancer, malnutrition, renal disease, renal failure, or any combination thereof. In other embodiments, the subject is suffereing from an irregular heartbeat; an exercise-induced irregular heartbeat; congestive heart failure; chronic obstructive pulmonary disease, high blood pressure or any combination thereof. In certain embodiments, the irregular heartbeat of the subject comprises atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPTV); and exercise-induced variants thereof. In certain embodiments, the exercise induced muscle fatigue is due to prolonged exercise. In other embodiments, the muscle fatigue is due to high-intensity-exercise.

[0025] In certain embodiments, the muscle fatigue is high-intensity-exercise-induced muscle fatigue in a subject. In another embodiment, the muscle fatigue is due to prolonged exercise. In other embodiments, the muscle fatigue is age-related muscle fatigue in a subject. In certain embodiment, muscle fatigue is associated with a disease or disorder. In certain embodiments, muscle fatigue is due to a myopathy. In other embodiments, the muscle fatigue is due to a muscular dystrophy in a subject.

[0026] In certain aspects, the invention provides a method for treating or preventing myopathy in a subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof. In certain aspects, the invention provides methods for treating or preventing muscular dystrophy in a subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof. In certain aspects, the invention provides methods for treating or preventing central core disease in a subject, the method

comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof. In certain aspects, the invention provides methods for treating or preventing malignant hyperthermia in a subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof. In certain embodiments, the compound is represented by the structure of Formula I-a, I-b, I-e, I-f, I-g, I-h, I-l, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof.

[0027] In other embodiments, the compound is selected from the group consisting of S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, and any combination thereof. In certain embodiments, the compound is not S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, or S100 as described herein. In certain embodiments, the compound is not S4, S7, S20, S24, S25, S26, S27, or S36. In another embodiment, the compound is not JTV-519.

[0028] In other embodiments, the methods comprise administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts,

hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof. In other embodiments, the compound is selected from the group consisting of S68, S101, S102, S103, S104, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, any salt, hydrate, solvate, polymorph, complex, metabolite, pro-drugs thereof, and any combination thereof. In other embodiment, the compound is selected from the group consisting of S101, S102, S103, S107, S109, S110, S111, S117, and S121, any salt, hydrate, solvate, polymorph, complex, metabolite, pro-drugs thereof, and any combination thereof. In other embodiments, the compound is S107, any salt, hydrate, solvate, polymorph, complex, metabolite, pro-drug thereof, or any combination thereof. In other embodiments, the compound is S117, any salt, hydrate, solvate, polymorph, complex, pro-drug thereof, or any combination thereof.

[0029] In certain embodiments of the methods of the present invention, the compounds are administered in as a specific salt, hydrate, solvate, isomer, enantiomer, or in a substantially pure form. In certain embodiments, wherein the compound S117 is administered, the compound is administered as an isolated compound in a substantially pure form. In certain embodiments of the methods, wherein a myopathy or muscular dystrophy is treated, the symptoms can be muscle fatigue, exercise-induces muscle fatigue, or any other symptoms associated with skeletal muscle defect.

[0030] In another aspect, the invention provides a method for reducing calpain activity, or plasma creatine kinase activity or level, in a subject so as to treat or reduce skeletal muscle fatigue in the subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I. In another aspect, the invention provides a method for reducing calpain activity, or plasma creatine kinase activity or level, in a subject so as to treat or reduce skeletal muscle damage in the subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I. In another aspect, the invention provides a method for reducing calpain activity, or plasma creatine kinase activity or level, in a subject so as to treat or reduce defective skeletal muscle function in the subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I. In another aspect, the invention provides a method for reducing calpain activity, or plasma creatine kinase activity or level, in a subject so as to treat or dealy the onset of myopathy, for example but not limited to central core disease, or

malignant hyperthermia, in the subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I. In another aspect, the invention provides a method for reducing calpain activity, or plasma creatine kinase activity or level, in a subject so as to treat or dealy the onset of muscular dystrophy in the subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I.

[0031] In other embodiments, the compound is represented by the structure of Formula I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof. In other embodiments, the compound is selected from the group consisting of S1, S2, S3, S5, S6, S9, S11, S12, S13, S14, S19, S22, S23, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, and any combination thereof. In other embodiments, the compound is selected from the group consisting of S101, S102, S103, S107, S109, S110, S111, S117, and S121. In one embodiment, the compound is S107 or a pharmaceutically acceptable salt thereof. In another embodiment, the compound is S117 or a pharmaceutically acceptable salt thereof.In certain embodiments, the compound is not S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, or S100. In another embodiment, the compound is not S4, S7, S20, S24, S25, S26, S27, or S36. In one embodiment, the compound is not JTV-519.

[0032] In certain embodiments of the methods which treat muscular dystrophy, the muscular dystrophy is selected from the group consisting of Duchenne muscular dystrophy, facioscapulohumeral dystrophy, limb girdle muscular dystrophy, myotonic muscular dystrophy, Becker's muscular dystrophy, congenital muscular dystrophy, distal muscular

dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy , myotonic muscular dystrophy, and oculopharyngeal muscular dystrophy .

[0033] In certain aspects, the invention provides methods for treatment, wherein the subject is a human. In certain embodiments, the subject is a non-human animal selected from the group consisting of: canine, equine, feline, porcine, murine, bovine, foul, sheep, primates, rodents, ovine and bovine.

[0034] In other aspects, the invention provides methods for treatment, wherein the compound is part of a pharmaceutical composition. In certain embodiments, the pharmaceutical composition comprises at least one pharmaceutically acceptable excipient. In other embodiment, the at least one pharmaceutically acceptable excipient comprises aromatics, buffers, binders, colorants, disintegrants, diluents, emulsifiers, extenders, flavor-improving agents, gellants, glidants, preservatives, skin-penetration enhancers, solubilizers, stabilizers, suspending agents, sweeteners, tonicity agents, vehicles, viscosity-increasing agents or any combination thereof. In other embodiments, the pharmaceutical composition further comprises a second active agent. In certain embodiments, the second active agent is an analgesic. In certain embodiments, the composition is in a capsule form, a granule form, a powder form, a solution form, a suspension form, or a tablet form, or in the form of an animal feed.

[0035] In certain embodiments, the composition is administered via parenteral, enteral, intravenous, intraarterial, intraspinal, intra, intraosseal, intracutaneous, subcutaneous, intradermal, subdermal, transdermal, intrathecal, intramuscular, intraperitoneal, intrasternal, parenchymatous, oral, sublingual, buccal, rectal, vaginal, inhalational, or intranasal administration. In certain embodiments, the compound is administered using a drug-releasing implant or via osmotic pump. In certain embodiments, the compound is administered to the subject at a dose sufficient to restore binding of calstabin 1 to RyR1. In certain embodiments, the compound is administered to the subject at a dose sufficient to enhance binding of calstabin 1 to RyR1. In certain embodiments, the compound is administered to the subject at a dose sufficient to reduce calpain activity. In certain embodiments, the compound is administered to the subject as a dose sufficient to reduce plasma creatine kinase activity or levels. In certain embodiments which treat stress or

exercise induced fatigue claims, administering of the compound begins prior to the beginning of (7, 6, 5, 4, 3, 2, 1 days), concurrently with the duration of, at end of , or after end of the exercise or stress condition (1, 2, 3, 4, 5 , 6, 7 days). In certain embodiments, the compound is administered to the subject at a dose of from about 0.01 mg/kg/day to about 20 mg/kg/day. In certain embodiments, the compound is administered to the subject at a dose of from about 0.05 mg/kg/day to about 1 mg/kg/day.

[0036] In certain aspects the invention provides methods for treating or preventing muscle fatigue in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases the open probability of the RyR1 channel. In certain embodiments, the invention provides a method of treating or preventing muscle fatigue in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases Ca2+ current through the RyR1 channel. In certain embodiments, the invention provides a method of treating or preventing muscle fatigue in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases calcium leak through the RyR1 channel. In other embodiments, the invention provides a method of treating or preventing muscle fatigue in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that increases the affinity with which calstabin 1 binds to RyR1. In other embodiments, the invention provides a method of treating or preventing muscle fatigue in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases dissociation of calstabin 1 from RyR1. In certain embodiments, the invention provides a method of treating or preventing muscle fatigue in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that increases rebinding of calstabin 1 to RyR1. In certain embodiments, the invention provides a method of treating or preventing muscle fatigue in a subject in need thereof, comprising, administering to the subject a therapeutically or prophylactically effective amount of a compound that mimics the binding of calstabin 1 to RyR1.

[0037] In certain embodiments of the methods of the present invention, the subject is suffering form a myopathy, a neurological disorder, an infectious disease, a chronic disorder , a genetic disease, or any other disorder or disease which is associated with symptoms of

muscle fatigue. Non-limiting examples of diseases or disorders are a cardiac disease or disorder, defective skeletal muscle function, HIV Infection, AIDS, muscular dystrophy, cancer, malnutrition, exercise-induced muscle fatigue, age-associated muscle fatigue, renal disease, renal failure, an irregular heartbeat; an exercise-induced irregular heartbeat; congestive heart failure; chronic obstructive pulmonary disease, high blood pressure or any combination thereof. The irregular heartbeat of the subject comprises atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPTV); and exercise-induced variants thereof.

[0038] In certain embodiments, the subject is a human. In other embodiments, the subject is a non-human animal such as a canine, equine, feline, porcine, murine, bovine, foul, sheep, or any other animal in need of treatment.

[0039] The invention relates to compositions and methods for treating muscle fatigue by administering novel compounds that stabilize ryanodine receptors (RyR), which regulate calcium channel functioning in cells. The invention provides methods for treating or preventing or reducing muscle fatigue by administering 1,4-benzothiazepine compounds that are RyR stabilizers. The muscle fatigue in a subject may be caused by a wide variety of acute and chronic pathologies, diseases or conditions, including but not limited to cardiac disease, HIV infection and IDS, muscular dystrophy, cancer, malnutrition, exercise-induced muscle fatigue, age-associated muscle fatigue, renal disease and renal failure. The invention provides for methods for treating or preventing ryanodine receptor calcium leak and muscle fatigue in subjects suffereing from a disease or disorder characterized by muscle fatigue, or in subjects suffering from muscle fatigue as a result of sustained, prolonged and/or strenuous exercise, or chronic stress. The invention provides methods for treating muscle fatigue with calcium release channel stabilizing drugs such RyCal compounds. The invention provides methods for reducing calpain activity or levels. The invention provides methods to reduce plasma creatine kinase activity.

[0040] The compounds used in the methods of the present invention modulate calcium-ion channels in cells of the subject. In some embodiments, the compounds used in the methods of the present invention decrease the release of calcium into cells of the subject. In other embodiments, the compounds used in the methods of the present invention limit or prevent a

decrease in the level of RyR-bound FKBP in the subject. In other embodiments, the compounds used in the methods of the present invention inhibit dissociation of FKBP and RyR in cells of the subject. In other embodiments, the compounds used in the methods of the present invention increase binding between FKBP and RyR in cells of the subject. In other embodiments, the compounds used in the methods of the present invention stabilize the RyR-FKBP complex in cells of a subject. In other embodiments, the compounds used in the methods of the present invention limit, prevent, or treat a leak in a RyR receptor in the subject. In other embodiments, the compounds used in the methods of the present invention modulate the binding of RyR and FKBP in the subject. In other embodiments, the compounds used in the methods of the present invention reduce the open probability of RyR by increasing the affinity of FKBP for PKA-phosphorylated RyR. In certain embodiments of the present invention RyR is RyR1. In certain embodiments of the present invention FKBP is calstabin1.

[0041] In certain embodiments, the methods for treatment of the present invention are directed to treatment of muscle fatigue which is due to sustained or strenuous exercise. In other embodiments, the methods for treatment of the present invention are directed to treatment of muscle fatigue which is due to chronic stress.

[0042] In certain embodiments, the present invention is directed to methods of treating or preventing muscle conditions, including but not limited to myopathies, such as muscular dystrophies. In certain embodiments, the present invention is directed to methods of treating myopathy, in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of one of the compounds of the invention, such as a compound of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes or pro-drugs thereof.

[0043] The methods and compositions of the invention may be used to treat any subject in need thereof. In certain embodiments, the subject is a mammal, such as a mammal selected from the group consisting of primates, rodents, ovine species, bovine species, porcine species, equine species, feline species and canine species. In preferred embodiments, the subject is a human.

[0044] The methods and compositions of the invention may be used to treat or prevent conditions, diseases and disorders affecting the muscles, such as myopathies. For example, the methods and compositions of the invention may be used to treat or prevent a myopathy selected from the group consisting of congenital myopathies, muscular dystrophies, mitochondrial myopathies, endocrine myopathies, muscular glycogen storage diseases, myoglobinurias, dermatomyositis, myositis ossificans, familial periodic paralysis, polymyositis, inclusion body myositis, neuromyotonia, and stiff-man syndrome. A non-limiting example of diseases or disorders which affect skeletal muscle functions is central core disease, malignant hyperthermia and the like

[0045] In preferred embodiments, the methods and compositions of the invention may be used to treat or prevent a muscular dystrophy. For example, the methods and compositions of the invention may be used to treat or prevent a muscular dystrophy selected from the group consisting of Duchenne muscular dystrophy, facioscapulohumeral dystrophy, limb girdle muscular dystrophy, myotonic muscular dystrophy, Becker's muscular dystrophy, congenital muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy , myotonic muscular dystrophy, and oculopharyngeal muscular dystrophy.

[0046] The compositions of the invention may be administered using any suitable method known in the art. For example, the compositions of the invention may be administered using a route selected from the group consisting of parenteral, enteral, intravenous, intraarterial, intraspinal, intra, intraosseal, intracutaneous, subcutaneous, intradermal, subdermal, transdermal, intrathecal, intramuscular, intraperitoneal, intrasternal, parenchymatous, oral, sublingual, buccal, rectal, vaginal, inhalational, and intranasal, or using drug-releasing implant. s of treating or preventing myopathy, in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of the compound S107, or an enantiomer, diastereomer, tautomer, pharmaceutically acceptable salt, hydrate, solvate, complex, or prodrug thereof.

[0047] In a further preferred embodiment, the present invention is directed to methods of treating or preventing muscular dystrophy, such as Duchenne muscular dystrophy, in a subject in need thereof, comprising administering to the subject a therapeutically or

prophylactically effective amount of the compound S107, or an enantiomer, diastereomer, tautomer, pharmaceutically acceptable salt, hydrate, solvate, complex, or prodrug thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0048] Figures 1A and 1B show tracking data during swimming on the first day of exercise for control vehicle treated (PBS) mice and mice treated with S36. Individual mouse velocities over five minute time intervals are shown in Figure 1A and mean velocities of each treatment group are shown in Figure 1B (n = 4 PBS, n = 4 S36). After one day of exercise, there is no substantial difference between the treatment groups. Fg-100, fg-103, fg-105, fg-106 indicates mice treated with S36. Fg-101, fg-102, fg-104, fg-107 indicates mice treated with PBS.

[0049] Figures 2A-2F show that there is no substantial difference between the different treatment groups after 7 days of exercise. Fig. 2A and 2B show results of swimming exercise for PBS and S36 treated mice. Individual mouse velocities over five minute time intervals are shown in Embodiment A and mean velocities of each treatment group are shown in Figure 2B (n = 3 PBS, n = 4 S36). Figures 2C-2F show results of a treadmill running exercise on day 7. As described, mice were run on the treadmill on an exercise protocol of increasing intensity, shown on the left marked velocity (m/min) in grey. Fig. 2C shows individual traces reflecting the number of visits to the shocking area at the rear of the treadmill over each three minute interval. Task failure can be clearly appreciated from the rapid rise in visits to the shocking area as the mice fail. Fig. 2D shows the number of shocks delivered to each mouse in each three minute interval, on an inverted axis, plotted as points with a three point moving average interpolation for each mouse. Fig. 2E depicts quantification of total distance run in meters before failure for PBS and S36 treatment groups (n = 3 PBS, n = 4 S36), and Embodiment F depicts quantification of fatigue times, defined as time to task failure, for PBS and S36 treatment groups. (n = 3 PBS, n = 4 S36). No significant difference is observed between the different treatment groups.

[0050] Figures 3A-3F show that after 14 days of swimming, there is no significant difference in the swimming velocities of the different treatment groups. Fig. 3 A shows individual mouse velocities, and Fig. 3 B shows mean velocities for each treatment group. n = 3 PBS, n = 4 S36. Treadmill running on day fourteen demonstrates a trend toward improved performance in S36 treated mice. An increasing intensity exercise protocol was used, shown

on the left marked velocity (m/min) in grey. Fig. 3 C shows individual traces reflecting the number of visits to the shocking area at the rear of the treadmill over each three minute interval. Fig. 3 D shows the number of shocks delivered to each mouse in each three minute interval, on an inverted axis, plotted as points with a three point moving average interpolation for each mouse. Fig. 3 E depicts quantification of total distance run in meters before failure for PBS and S36 treatment groups. (n = 3 PBS, n = 4 S36), and Fig. 3 F depicts quantification of fatigue times, defined as time to task failure, for PBS and S36 treatment groups. (n = 3 PBS, n = 4 S36).

[0051] Figures 4 A, B, C and D show analysis of data on day 16 of exercise regimen. Figs. 4A and 4B shows results of treadmill running on day sixteen that replicates the trend toward improved performance in S36 treated mice. An increasing intensity exercise protocol was used, shown on the left marked velocity (m/min) in black. Fig. 4 A shows individual traces, which reflect the number of visits to the shocking area at the rear of the treadmill over each three-minute interval. Fig. 4 B shows the number of shocks delivered to each mouse in each three minute interval, on an inverted axis, plotted as points with a three point moving average interpolation for each mouse. Fig. 4 C shows quantification of total distance run in meters before failure for PBS and S36 treatment groups. (n = 3 PBS, n = 4 S36) Fig. 4D) shows quantification of fatigue times, defined as time to task failure, for PBS and S36 treatment groups. (n = 3 PBS, n = 4 S36). Figure 4 shows that the trend toward improved performance in S36 treated mice continues on page 16.

[0052] Figure 5 shows percent improvements in fatigue times and distance run of S36 treated mice compared to PBS vehicle treated mice under the same conditions at each day, as measured by the treadmill assay.

[0053] Figure 6 shows that in vivo treatment with S36 (ARM036) allows RyR1 to rebind calstabin1 despite intense chronic exercise. RyR1 was immunoprecipitated from soleus muscle homogenates of mice following 21 days of exercise with or without simultaneous subcutaneous mini-osmotic pump treatment with S36 and then western blotted back for RyR1, phospho-epitope specific RyR1-pS2844, and calstabin1 bound to the RyR1 macromolecular complex.

[0054] Figures 7C and 7D provide data illustrating that the RyCal compound S107 reduces calpain activity in the mdx mouse muscular dystrophy model during exercise, and indicates

that RyCals are useful for treating muscle related diseases, such as muscular dystrophies. Figs. 7A and 7B are intentionally left out.

[0055] Figures 8A – 8D show that the RyR1 Macromolecular Complex Undergoes Substantial Remodeling Following Exercise. Fig. 8A) Composition of the RyR1 complex in extensor digitorum longus (EDL) muscle following an exercise protocol (consisting of twice daily swimming) lasting the indicated number of days by immunoprecipitation of RyR1 and immunoblot for RyR, RyR1-pS2844, and PDE4D3 and calstabin1 bound to the receptor. Fig. 8B) Densitometric quantification of A, where each value is relative to the total RyR1 immunoprecipitated. Fig. 8C) Composition of the RyR1 complex in EDL muscle following low intensity and high intensity exercise for 5 days. Fig. 8D) Densitometric quantification of 8C. In all cases, the product of a single RyR1 immunoprecipitation was separated on a 4-20% gradient polyacrylamide gel, transferred, and probed for both total RyR1 and one or more of the modifications noted. The blots shown are representative of three independent experiments.

[0056] Figures 9A-9D show that high intensity cycling exercise in humans results in PKA phosphorylation of RyR1, and calstabin1 and PDE4D3 depletion. Fig. 9A) Immunoblot of the RyR1 complex immunoprecipitated from 100 ug of muscle homogenate from human thigh biopsies before and after exercise on day 1 and day 3 (Fig. 9C) of a high intensity (three hours at 57% VO_2 max) cycling protocol. Control cyclists sat in the exercise room but did not exercise. Immunoblots show total RyR1, RyR1-S2844 PKA phosphorylation, bound calstabin1, and bound PDE4D3. Fig. 9B) and Fig. 9D) Quantification by densitometry of Fig. 9A) and Fig. 9C) respectively. Bar graphs depict PKA phosphorylation, calstabin1, and PDE4D3 levels in the RyR1 complex normalized to total RYR1 from control (n = 6) and exercise (n = 12) biopsies on each day.

[0057] Figures 10A-10F show that Muscle-specific Cal1 -/- Mice Have a High Intensity Exercise Defect. Fig. 10A) Time to failure during a single level treadmill assay on 2 month-old cal1 -/- mice and w.t. littermates. Fig. 10B) Individual treadmill failure times for each mouse separated by gender. Fig. 10C) Body weights of the cal1 -/- mice were reduced. Fig. 10D) Scatter plot of failure time versus body weight shows no correlation in either group of mice. Fig. 10E) Plasma creatine kinase (CPK) levels at baseline and following a single downhill eccentric treadmill run. Fig. 10F) RyR1 immunoprecipitated

from EDL and immunoblotted for RyR, RyR1-pS2844, PDE4D3, and calstabin1. *, $p < 0.01$, Wilcoxon rank-sum test.

[0058] Figures 11A-11F shows that PDE4D -/- mice have an exercise defect. 11A) Time to failure during a single level treadmill assay on 2 month-old PDE4D -/- mice and w.t. littermates. 11B) Individual treadmill failure times for each mouse. 11C) Body weights of the PDE4D -/- mice. 11D) Scatter plot of failure time versus body weight shows no correlation in either group of mice. 11E) Plasma creatine kinase (CPK) levels at baseline and following a single downhill eccentric treadmill run. 11F) RyR1 immunoprecipitated from EDL and immunoblotted for RyR, RyR1-pS2844, PDE4D3, and calstabin1. *, $p < 0.05$, Wilcoxon rank-sum test.

[0059] Figures 12A-12F show that pharmacologic rebinding of calstabin1 to RyR1 improves in vivo exercise performance. Fig. 12A) Time to failure during treadmill assays on indicated days of a 28 day treatment trial with S107. Fig. 12B) Individual treadmill failure times for each mouse on Day 21. Fig. 12C) Force-frequency curves of EDL muscle isolated immediately following the 21st day of exercise and isometrically stimulated in an oxygenated muscle bath. Forces (cN) are normalized to muscle cross sectional area. Fig. 12D) Body weights throughout the trial showed no treatment effect. Fig. 12E) Treadmill failure times from a parallel experiment in muscle-specific cal1 -/- mice. Fig. 12F) RyR1 immunoprecipitated from EDL and immunoblotted for RyR, RyR1-pS2844, PDE4D3, and calstabin1. *, $p < 0.01$, Wilcoxon rank-sum test.

[0060] Figures 13A-13C show that chronic S107 treatment reduces fatiguability of isolated FDB fibers. Fig. 13A) Representative trace from a vehicle treated FDB fiber of fluo-4 fluorescence ($\Delta F/FO$) normalized to the peak during repeated 300 ms long, 120 Hz field-stimulated tetani at a train rate of 0.5. Hz. Isolated cells were continuously perfused with a HEPES buffered Tyrodes solution at room temperature. Fig. 13B) Representative tetanic trace from an S107 treated FDB fiber. Fig. 13C) Mean peak tetanic calcium, as measured by fluo-4 fluorescence ($\Delta F/FO$) normalized to the peak during fatiguing stimulation (n=11 vehicle, n=13 S107). *, $p< 0.02$ unpaired t-test.

[0061] Figures 14A-14B show that RyR1 from exercised muscle is leaky, with increased Po at resting calcium. (Fig. 14A) Representative traces of RyR1 channel activity at 90 nM [Ca2+]*cis* from sedentary mice (sed, *left column),* mice chronically exercised and treated with

vehicle (Ex + veh, *middle column)* and with S107 (Ex + S107, *right column).* Single channel openings are plotted as upward deflections; the open and closed (c) states of the channel are indicated by horizontal bars at the beginning of the traces. Corresponding channel open probability (Po), mean open time (To) and frequency of openings (Fo) are shown above each group of traces and represent average values from all experiments. (Fig. 14B) Average values of open probability *(left),* mean open times *(middle)* and frequency of openings *(right)* of RyR1 activity from sedentary mice (sed, n = 9) and mice chronically exercised treated either with vehicle (Ex + veh, n = 9) or S107 (Ex + S107, n = 12). Error bars indicate SEM; *, $p < 0.005$ compared to sed; #, $p < 0.005$ compared to Ex + S107.

[0062] Figures 15A-15B showsthat S107 protects against chronic exercise-induced muscle damage and calpain activation. Fig. 15A) Plasma creatine kinase (CPK) activity levels in sedentary and chronically exercised mice with, and without, calstabin1 rebinding with S107. Fig. 15B) Calpain activity levels in EDL homogenates measured using a fluorogenic calpain substrate assay. *, $p< 0.01$ unpaired t-test, S107 vs vehicle.

[0063] Figures 16A-16C show the effect of exercise on the composition of the RyR1 complex.

[0064] Figures 17A-17B show the distribution of 50% reuptake times (tau) in muscle fibers in the presence or absence of S107 treatment.

[0065] Figures 18A-18C show the progressive phosphorylation of RyR1 and calstabin 1 depletion from the RyR1 complex in an *mdx* mouse model as a factor of time.

[0066] Figures 19A-19F show the effect of S107 on exercise tolerance , body weight, CPK and calpain levels in wt (unaffected) and *mdx* mice.

[0067] Figure 20 shows histological slides of wt (unaffected) and *mdx* mice which are untreated or treated with S107.

DETAILED DESCRIPTION OF THE INVENTION

[0068] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "an agent" includes a plurality of such agents and equivalents thereof known to those skilled in the art, and reference to "the FKBP12.6 polypeptide" is a reference to one or

more FKBP12.6 polypeptides (also known as calstabin2) and equivalents thereof known to those skilled in the art, and so forth. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

[0069] As used herein the term "fatigue" refers to skeletal muscle fatigue and/or weakness. Muscle fatigue can be due to strenuous or repeated physical activity or exercise, chronic stress, disease, disorder, syndrome or any other underlying pathophysiological condition that has symptoms of fatigue, or affects myofibers and/or muscle function. Muscle fatigue is defined as the failure of exercise performance - this can be assessed on an exercise stress test and quantified as the time it takes to fail at the given task (e.g. walking/jogging/running on a treadmill). Failure at the task is defined as termination of the exercise due to inability to continue - this is defined as muscle fatigue.

[0070] Sustained or prolonged exercise is defined as exercise performed over a defined and measurable time period.

[0071] Strenuous exercise is exercise to evoke muscle fatigue within a defined time period.

[0072] Chronic stress is defined as conditions that cause muscle fatigue chronically either due to persistent chronic exercise or stress due to chronic diseases/disorders that are often associated with chronic activation of the sympathetic nervous systems (e.g chronic activation of the "fight or flight " response). In one embodiment, the subject's defective skeletal muscle function occurs during chronic obstructive pulmonary disease, hypertension, asthma, or hyperthyroidism.

[0073] In certain aspects, the present invention is directed to compositions and methods for the treatment and prevention of myopathies. The term "myopathy" as used herein refers to neuromuscular disorders caused by dysfunction in the muscle itself. The term "myopathy", as used herein, encompasses all of the myopathies described herein and also all other myopathies known to those of skill in the art.

[0074] Myopathies may be inherited (such as many of the muscular dystrophies) or acquired. Myopathic diseases and disorders include, but are not limited to, congenital myopathies, muscular dystrophies (characterized by progressive weakness in voluntary muscles), mitochondrial myopathies, endocrine myopathies, muscular glycogen storage diseases, myoglobinurias, dermatomyositis, myositis ossificans, familial periodic paralysis,

polymyositis, inclusion body myositis, neuromyotonia, stiff-man syndrome, common muscle cramps, and tetany.

[0075] Examples of muscular dystrophies include, but are not limited to, Duchenne muscular dystrophy, facioscapulohumeral dystrophy, limb girdle muscular dystrophy, and myotonic muscular dystrophy, Becker's muscular dystrophy, congenital muscular dystrophy, Distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, Facioscapulohumeral muscular dystrophy , Limb-girdle muscular dystrophy , Myotonic muscular dystrophy, and Oculopharyngeal muscular dystrophy. Examples of mitochondrial myopathies include, but are not limited to, Kearns-Sayre syndrome, MELAS and MERRF. MELAS which is an abbreviation of "mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke" is a progressive neurodegenerative disorder. MELAS affects multiple organ systems including the central nervous system (CNS), skeletal muscle, the eye, cardiac muscle, and, more rarely, the gastrointestinal and renal systems. MERFF, which is an abbreviation of "myoclonus epilepsy with ragged-red fibers" may cause epilepsy, coordination loss, dementia and muscle weakness. Examples of glycogen storage diseases of muscle include, but are not limited to, Pompe's disease, Andersen's disease, and Cori's diseases. Examples of myoglobinurias include, but are not limited to McArdle's disease, Tarui disease, and DiMauro disease.

[0076] The following are definitions of terms used in the present specification. The initial definition provided for a group or term herein applies to that group or term throughout the present specification individually or as part of another group, unless otherwise indicated.

[0077] As used herein, the term "RyCal compounds" refers to compounds of the general Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or II as provided by the invention, and herein referred to as "compound(s) of the invention".

[0078] The compounds of the invention are referred using a numerical naming system, with compound numbers 1 to 123 provided herein. These numbered compounds are referred to using either the prefix "S" or the prefix "ARM." Thus, the first numbered compound is referred to either as "S1" or "ARM001", the second numbered compound is referred to as either "S2" or "ARM002", the third numbered compound is referred to as either "S3" or "ARM003", and so on. The "S" and the "ARM" nomenclature systems are used interchangeably throughout the specification, the drawings, and the claims.

[0079] The term "alkyl" as used herein refers to a linear or branched, saturated hydrocarbon having from 1 to 6 carbon atoms. Representative alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, isohexyl, and neohexyl. The term "C_1-C_4 alkyl" refers to a straight or branched chain alkane (hydrocarbon) radical containing from 1 to 4 carbon atoms, such as methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, and isobutyl.

[0080] The term "alkenyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon double bond. In one embodiment, the alkenyl has one or two double bonds. The alkenyl moiety may exist in the E or Z conformation and the compounds of the present invention include both conformations.

[0081] The term "alkynyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon triple bond.

[0082] The term "aryl" as used herein refers to an aromatic group containing 1 to 3 aromatic rings, either fused or linked.

[0083] The term "cyclic group" as used herein includes a cycloalkyl group and a heterocyclic group.

[0084] The term "cycloalkyl group" as used herein refers to a three- to seven-membered saturated or partially unsaturated carbon ring. Any suitable ring position of the cycloalkyl group may be covalently linked to the defined chemical structure. Exemplary cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

[0085] The term "halogen" as used herein refers to fluorine, chlorine, bromine, and iodine.

[0086] The term "heterocyclic group" or "heterocyclic" or "heterocyclyl" or "heterocyclo" as used herein refers to fully saturated, or partially or fully unsaturated, including aromatic (i.e., "heteroaryl") cyclic groups (for example, 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 16 membered tricyclic ring systems) which have at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, 3, or 4 heteroatoms selected from nitrogen atoms, oxygen atoms and/or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. The heterocyclic group may be

attached to the remainder of the molecule at any heteroatom or carbon atom of the ring or ring system. Exemplary heterocyclic groups include, but are not limited to, azepanyl, azetidinyl, aziridinyl, dioxolanyl, furanyl, furazanyl, homo piperazinyl, imidazolidinyl, imidazolinyl, isothiazolyl, isoxazolyl, morpholinyl, oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, piperazinyl, piperidinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazolyl, pyridoimidazolyl, pyridothiazolyl, pyridinyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, quinuclidinyl, tetrahydrofuranyl, thiadiazinyl, thiadiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiomorpholinyl, thiophenyl, triazinyl, and triazolyl. Exemplary bicyclic heterocyclic groups include indolyl, isoindolyl, benzothiazolyl, benzoxazolyl, benzoxadiazolyl, benzothienyl, quinuclidinyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, benzofurazanyl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl] or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), triazinylazepinyl, tetrahydroquinolinyl and the like. Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthrolinyl, acridinyl, phenanthridinyl, xanthenyl and the like.

[0087] The term "phenyl" as used herein refers to a substituted or unsubstituted phenyl group.

[0088] The aforementioned terms “alkyl,” “alkenyl," “alkynyl,” “aryl,” “phenyl,” “cyclic group,” “cycloalkyl,” “heterocyclyl,” “heterocyclo,” and “heterocycle” may further be optionally substituted with one or more substituents. Exemplary substituents include but are not limited to one or more of the following groups: hydrogen, halogen, CF_3, OCF_3, cyano, nitro, N_3, oxo, cycloalkyl, alkenyl, alkynyl, heterocycle, aryl, alkylaryl, heteroaryl, OR_a, SR_a, $S(=O)R_e$, $S(=O)_2R_e$, $P(=O)_2R_e$, $S(=O)_2OR_a$, $P(=O)_2OR_a$, NR_bR_c, $NR_bS(=O)_2R_e$, $NR_bP(=O)_2R_e$, $S(=O)_2NR_bR_c$, $P(=O)_2NR_bR_c$, $C(=O)OR_a$, $C(=O)R_a$, $C(=O)NR_bR_c$, $OC(=O)R_a$, $OC(=O)NR_bR_c$, $NR_bC(=O)OR_a$, $NR_dC(=O)NR_bR_c$, $NR_dS(=O)_2NR_bR_c$, $NR_dP(=O)_2NR_bR_c$, $NR_bC(=O)R_a$, or $NR_bP(=O)_2R_e$, wherein R_a is hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl; R_b, R_c and R_d are independently hydrogen, alkyl, cycloalkyl, alkylaryl, heteroaryl, heterocycle, aryl, or said R_b and R_c together with the N to which they are bonded optionally form a heterocycle; and R_e is alkyl, cycloalkyl, alkenyl,

cycloalkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl. In the aforementioned exemplary substitutents, groups such as alkyl, cycloalkyl, alkenyl, alkynyl, cycloalkenyl, alkylaryl, heteroaryl, heterocycle and aryl can themselves be optionally substituted.

[0089] Exemplary substituents may further optionally include at least one labeling group, such as a fluorescent, a bioluminescent, a chemiluminescent, a colorimetric and a radioactive labeling group. A fluorescent labeling group can be selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof. For example, ARM118 of the present invention contains a labeling group BODIPY, which is a family of fluorophores based on the 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene moiety. For further information on fluorescent label moieties and fluorescence techniques, see, e.g., *Handbook of Fluorescent Probes and Research Chemicals*, by Richard P. Haughland, Sixth Edition, Molecular Probes, (1996), which is hereby incorporated by reference in its entirety. One of skill in the art can readily select a suitable labeling group, and conjugate such a labeling group to any of the compounds of the invention, without undue experimentation.

[0090] The term "quaternary nitrogen" refers to a tetravalent positively charged nitrogen atom including, for example, the positively charged nitrogen in a tetraalkylammonium group (e.g., tetramethylammonium, N-methylpyridinium), the positively charged nitrogen in protonated ammonium species (e.g., trimethyl-hydroammonium, N-hydropyridinium), the positively charged nitrogen in amine N-oxides (e.g., N-methyl-morpholine-N-oxide, pyridine-N-oxide), and the positively charged nitrogen in an N-amino-ammonium group (e.g., N-aminopyridinium).

[0091] Throughout the specification, unless otherwise noted, the nitrogen in the benzothiazepine ring of compounds of the present invention may optionally be a quaternary nitrogen. Non-limiting examples include ARM-113 and ARM-119.

[0092] Compounds of the present invention may exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present invention.

[0093] The term "prodrug" as employed herein denotes a compound that, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield compounds of the present invention. A "pro-drug" refers to an agent which is converted into the parent drug *in vivo*. Pro-drugs are often useful because, in some situations, they are easier to administer than the parent drug. They are bioavailable, for instance, by oral administration whereas the parent drug is not. The pro-drug also has improved solubility in pharmaceutical compositions over the parent drug. For example, the compound carries protective groups which are split off by hydrolysis in body fluids, *e.g.*, in the bloodstream, thus releasing active compound or is oxidized or reduced in body fluids to release the compound.

[0094] All stereoisomers of the compounds of the present invention (for example, those which may exist due to asymmetric carbons on various substituents), including enantiomeric forms and diastereomeric forms, are contemplated within the scope of this invention. Individual stereoisomers of the compounds of the invention may, for example, be substantially free of other isomers (e.g., as a pure or substantially pure optical isomer having a specified activity), or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the present invention may have the S or R configuration as defined by the IUPAC 1974 Recommendations. The racemic forms can be resolved by physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomeric derivatives or separation by chiral column chromatography. The individual optical isomers can be obtained from the racemates by any suitable method, including without limitation, conventional methods, such as, for example, salt formation with an optically active acid followed by crystallization.

[0095] Compounds of the present invention are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an amount by weight equal to or greater than 99% of the compound ("substantially pure" compound), which is then used or formulated as described herein. Such "substantially pure" compounds of the present invention are also contemplated herein as part of the present invention.

[0096] All configurational isomers of the compounds of the present invention are contemplated, either in admixture or in pure or substantially pure form. The definition of

compounds of the present invention embraces both cis (*Z*) and trans (*E*) alkene isomers, as well as cis and trans isomers of cyclic hydrocarbon or heterocyclic rings.

[0097] Metabolite as used herein refers to a byproduct produced in vivo, for example in a subject, from a chemical compound.

[0098] Throughout the specifications, groups and substituents thereof may be chosen to provide stable moieties and compounds.

[0099] As used herein, the term "RyCal compounds" refers to compounds of the general Formula I, I-a (Ia), I-b (Ib), I-c (Ic), I-d (Id), I-e (Ie), I-f (If), I-g (Ig), I-h (Ih), I-i (Ii), I-j (Ij), I-k (Ik), I-l (Il), I-m (Im), I-n (In), I-o (Io), I-p (Ip) or II as provided by the invention, and herein referred to as compound(s) of the invention. Such compounds include, but are not limited to, any one or more of the compounds of formulae including, but not limited to, the compounds of formulae S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99,S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, as herein defined. In certain embodiments, the compounds are isolated and substantially pure.

[00100] A subject treated by the methods of the invention can include a mammal. Such a mammal can include a human, primate, canine, equine, feline, porcine, murine, bovine, foul, ungulate or sheep. The terms "animal," "subject" and "patient" as used herein include all members of the animal kingdom including, but not limited to, mammals, animals (e.g., cats, dogs, horses, etc.) and humans.

[00101] "PKA phosphorylation" means a reaction in which a phosphate group is substituted for a hydroxyl group by the enzyme protein kinase A (PKA).

[00102] "Back-phosphorylation" of RyR1 or RyR2 receptor means the in vitro phosphorylation of receptor by protein kinase A.

[00103] A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or pharmaceutically acceptable salts, hydrates, polymorphs, or pro-drugs thereof, with other chemical components, such as physiologically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[00104] A compound of the present invention also can be formulated as a pharmaceutically acceptable salt, *e.g.*, acid addition salt, and complexes thereof. The preparation of such salts can facilitate the pharmacological use by altering the physical characteristics of the agent without preventing its physiological effect. Examples of useful alterations in physical properties include, but are not limited to, lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate administering higher concentrations of the drug.

[00105] The term "pharmaceutically acceptable salt" means a salt which is suitable for or compatible with the treatment of a patient or a subject such as a human patient or an animal.

[00106] The term "pharmaceutically acceptable acid addition salt" as used herein means any non-toxic organic or inorganic salt of any base compounds represented by Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p, or Formula II, any of their intermediates. Illustrative inorganic acids which form suitable acid addition salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable acid addition salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed and such salts exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of the invention are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms.

[00107] A compound of the present invention also can be formulated as a pharmaceutically acceptable salt, *e.g.*, acid addition salt, and complexes thereof. The

preparation of such salts can facilitate the pharmacological use by altering the physical characteristics of the agent without preventing its physiological effect. Examples of useful alterations in physical properties include, but are not limited to, lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate administering higher concentrations of the drug.

[00108] The term "pharmaceutically acceptable salt" means a salt that is suitable for, or compatible with, the treatment of a patient or a subject such as a human patient. The salts can be any non-toxic organic or inorganic salt of any of the compounds represented by Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p or any of the specific compounds described herein, or any of their intermediates. Illustrative salt-forming ions include, but are not limited to, ammonium (NH_4^+), calcium (Ca^{2+}), iron (Fe^{2+} and Fe^{3+}), magnesium (Mg^{2+}), potassium (K^+), pyridinium ($C_5H_5NH^+$), quaternary ammonium (NR_4^+), sodium (Na^+), acetate, carbonate, chloride, bromide, citrate, cyanide, hydroxide, nitrate, nitrite, oxide, phosphate, sulfate, maleate, fumarate, lactate, tartrate, gluconate, besylate, and valproate. Illustrative inorganic acids that form suitable salts include, but are not limited to, hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable acid addition salts include, but are not limited to, mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either mono or di-acid salts can be formed, and such salts exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of an appropriate salt can be performed by one skilled in the art. For example, one can select salts in reference to "Handbook of Pharmaceutical Salts : Properties, Selection, and Use" by P. Heinrich Stahl and Camille G. Wermuth, or Berge (1977) "Pharmaceutcial Salts" J. Pharm Sci., Vol 66(1), p 1-19. Other non-pharmaceutically acceptable salts (*e.g.*, oxalates) may be used, for example, in the isolation of compounds of the invention for laboratory use or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

[00109] The compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, and I-p, and Formula II of the present invention may form hydrates or solvates, which are included in the scope of the claims. When the compounds of Formula I of the present invention exist as regioisomers, configurational isomers, conformers or diasteroisomeric forms all such forms and various mixtures thereof are included in the scope of Formula I. It is possible to isolate individual isomers using known separation and purification methods, if desired. For example, when a compound of Formula I of the present invention is a racemate, the racemate can be separated into the (S)-compound and (R)-compound by optical resolution. Individual optical isomers and mixtures thereof are included in the scope of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, and I-p, and Formula II.

[00110] The term "solvate" as used herein means a compound of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, and I-p, and Formula II or a pharmaceutically acceptable salt of a compound of Formula I, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate."

[00111] The term "polymorph" refers to a particular crystalline state of a substance, having particular physical properties such as X-ray diffraction, IR spectra, melting point, and the like.

[00112] The term an "effective amount," "sufficient amount" or "therapeutically effective amount" of an agent as used herein is that amount sufficient to effect beneficial or desired results, including clinical results and, as such, an "effective amount" depends upon the context in which it is being applied. The response is preventative and/or therapeutic. The term "effective amount" also includes that amount of the compound of Formula I which is "therapeutically effective" and which avoids or substantially attenuates undesirable side effects.

[00113] As used herein and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (*i.e.*, not worsening)

state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

[00114] As used herein, the term "inhibiting dissociation" includes blocking, decreasing, inhibiting, limiting or preventing the physical dissociation or separation of an FKBP subunit from an RyR molecule in cells of the subject, and blocking, decreasing, inhibiting, limiting or preventing the physical dissociation or separation of an RyR molecule from an FKBP subunit in cells of the subject.

[00115] As used herein, the term "increasing binding" includes enhancing, increasing, or improving the ability of phosphorylated RyR to associate physically with FKBP (*e.g.*, binding of approximately two fold or, approximately five fold, above the background binding of a negative control) in cells of the subject and enhancing, increasing or improving the ability of FKBP to associate physically with phosphorylated RyR (*e.g.*, binding of approximately two fold, or, approximately five fold, above the background binding of a negative control) in cells of the subject.

[00116] As used herein, the term "cardiac muscle cell" includes cardiac muscle fibers, such as those found in the myocardium of the heart.

[00117] The present invention provides compounds that are capable of treating and preventing disorders and diseases associated with the RyR receptors that regulate calcium channel functioning in cells. More particularly, the present invention provides compounds that are capable of treating or preventing a leak in RyR channels. "Disorders and diseases associated with the RyR receptors" means disorders and diseases that can be treated and/or prevented by modulating the RyR receptors that regulate calcium channel functioning in cells. "Disorders and diseases associated with the RyR receptors" include, without limitation, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, central core disease, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and

diseases include and exercise-induced irregular heartbeat disorders and diseases include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss.

[00118] As contemplated herein, the compounds of the invention are capable of treating and preventing disorders and diseases associated with the RyR receptors that regulate calcium channel functioning in cells, by repairing the leak in RyR channels, and enhancing binding of FKBP proteins (e.g., calstabin1) to PKA-phosphorylated RyR. Thus, in one embodiment, the compounds are useful to prevent and treat muscle fatigue that is associated with the RyR receptors that regulate calcium channel functioning in cells.

[00119] In one embodiment, the compounds of the invention are effective to treat muscle fatigue that results from pathologies, illnesses, diseases, disorders or conditions that are associated with the RyR receptors that regulate calcium channel functioning in cells. Examples of such disorders and conditions include, but are not limited to, cardiac disease or disorder, defective skeletal muscle function, HIV Infection, AIDS, muscular dystrophy, cancer, malnutrition, exercise-induced muscle fatigue, age-associated muscle fatigue, renal disease, and renal failure.

[00120] Examples of cardiac disorders and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Examples of irregular heartbeat disorders and diseases and exercise-induced irregular heartbeat disorders and diseases include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPTV); and exercise-induced variants thereof.

[00121] In one embodiment, the compounds of the invention modulate calcium-ion channels in cells of the subject. In another embodiment, the compounds of the invention

decrease the release of calcium into cells of the subject. In another embodiment, the compounds of the invention limit or prevent a decrease in the level of RyR-bound FKBP in the subject. In another embodiment, the compounds of the invention inhibit dissociation of FKBP and RyR in cells of the subject. In another embodiment, the compounds of the invention increase binding between FKBP and RyR in cells of the subject. In another embodiment, the compounds of the invention stabilize the RyR-FKBP complex in cells of a subject. In another embodiment, the compounds of the invention prevent, or treat a leak in a RyR receptor in the subject. In another embodiment, the modulate the binding of RyR and FKBP in the subject. In another embodiment, the compounds of the invention reduce the open probability of RyR by increasing the affinity of FKBP for PKA-phosphorylated RyR. In another embodiment, the compounds of the invention reduce or inhibit calpain activity so as to treat muscle fatigue. In another embodiment, the compounds of the invention reduce plasma creatine kinase levels so as to treat muscle fatigue.

[00122] The methods of the present invention can be practiced *in vitro* or *in vivo*. Thus, in one embodiment, the methods of the present invention are practiced in an *in vitro* system (*e.g.*, in a test tube on isolated cellular components). In another embodiment, the methods of the invention are practiced *in vivo*, *e.g.*, in cultured cells or tissues, or in subjects.

[00123] In another embodiment, the present invention provides use of a compound represented by the structure of formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, and I-p, and Formula II in the preparation of a medicament for treating or preventing muscle disorder or disease, for example but not limited to muscle fatigue in a subject.

[00124] In another embodiment, muscle fatigue can be caused by increased stress such as in individuals exposed to a continued and prolonged exercise regimen, e.g., soldiers or athletes. Thus in one embodiment, the compounds of the invention are useful for treating muscle fatigue in individuals exposed to stress due to, for example, an intense exercise regimen. Skeletal muscular disorder and diseases include, but are not limited to, stress induced skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence.

[00125] Pending applications with USSN 11/212,309 and 11/212,413, and PCT application PCT/US2006/32405 teach synthesis of small lead compounds on the basis of 1,4-

benzothiazepines which rescue RyR1 channel function and Ca^{2+} leak by increasing calstabin1 binding..

[00126] **Skeletal Muscle Fatigue**

[00127] Defects in $Ca2^{+}$ release channel, for example increased "leakiness" of the channel can lead to skeletal muscle fatigue. In certain aspects the invention provides that RyCal compounds which treat defects in $Ca2^{+}$ release channel can be used in methods for treating, reducing or preventing muscle disorders, muscle fatigue, including but not limited to exercise-induced muscle fatigue or muscle damage, muscle fatigue or damage associated with a disease condition, for example but not limited to a myopathy, muscular dystrophy and the like. Recent studies suggesting that lactic acid accumulation may not be detrimental have raised questions about the molecular basis underlying skeletal muscle fatigue. Among hypotheses a role for defective regulation of calcium has been proposed.

[00128] The invention provides data showing altered function of the major calcium release channel in skeletal muscle sarcoplasmic reticulum (SR), the type 1 ryanodine receptor (RyR1), is required for excitation-contraction coupling (ECC), during chronic exercise. During chronic exercise the RyR1 channel is PKA hyperphosphorylated at Ser2844 (Ser2843 in human). The PKA hyperphosphorylation is associated with depletion of the phosphodiesterase PDE4D3 from the RyR1 complex. Furthermore, PKA hyperphosphorylation contribute to depletion of the RyR1 stabilizing subunit calstabin1 (FKBP12) from the channel macromolecular complex resulting in "leaky" channels (increased open probability under conditions when normal channels are not active). The degree of PKA phosphorylation, and depletion of calstabin1 and PDE4D3 are correlated with the intensity and duration of exercise and progressive fatigue. Mice with skeletal muscle-specific calstabin1 deficiency and PDE4D deficient mice both exhibited significantly impaired exercise capacity. A small molecule, S107, that specifically causes rebinding of calstabin1 to the RyR1 channel improved exercise capacity and force generation of isolated muscle during a 21 day exercise protocol. S107 treated muscle fibers exhibited reduced fatigue, as determined by measurement of intracellular calcium during repeated tetanic contractions. Furthermore, S107 treated chronically exercised mice exhibited reduced levels of plasma creatine kinase, and calcium-dependent neutral protease calpain activity in muscle homogenates. This demonstrates the existence of

a mechanism of muscle fatigue, during chronic or high-intensity exercise, where SR calcium leak due calstabin1 depleted RyR1 channels leads to defective calcium signaling and skeletal muscle damage. In one aspect, the invention provides use of RyCal compounds which target molecular mechanisms of muscle fatigue, muscle conditions and disorders, and provide treatments treatment thereof.

[00129] Calcium release channel stabilizing drugs prevent muscle fatigue by preventing ryanodine receptor calcium leak during sustained or strenuous exercise. Repeated and strenuous activity of skeletal muscle may cause 1) weakness with intense use (also referred to as fatigue), 2) feeling of sore and weak muscles (referred to as perception), and 3) different degrees of muscle degeneration (referred to as dystrophic remodeling). A dominant theory of muscle fatigue has been that accumulation of intracellular lactic acid resulting in intracellular acidosis directly inhibits force production by the myofibrillar proteins (Hill et al., 1929). Indeed, at lower than physiologic temperatures (20°C), acidotic changes of intracellular pH were found to accelerate fatigability of skeletal muscles (Hill at al., 1929). However, more recent studies have challenged the significance of acidosis for muscle fatigue by showing that repeated short tetanic contractions which induce fatigue do not result in significant intracellular pH changes under more physiologic conditions at 37°C (Westerblad at al., 1997) where acidosis does not significantly effect force production. These findings are consistent with lactic acid which is produced during fatiguing contractions being extruded at a substantial rate by lactate transporters. However, during very intense athletic training lactic acid resulting from anaerobic breakdown of glycogen remains an important limiting factor. Importantly, intracellular acidosis was found to preserve muscle excitability and relaxation of the myofilaments during sustained increases of intracellular Ca^{2+} during repeated or sustained tetanic contractions, and thus protects from muscle fatigue (Pedersen at al. 2004).

[00130] Given that changes in intracellular pH may not represent a major fatigue mechanism, it is likely that alterations in EC coupling contribute to fatigue. Intracellular Ca^{2+} release via RyR1 channels initiates muscle contraction. Now classic physiologic experiments suggested in 1963, that reversible alterations in contractile activation may play an important role in muscle fatigue (Eberstein et al., 1963). During progressive development of fatigue resulting from repeated tetanic contractions, elevations in intracellular Ca^{2+} concentrations decline which explains reduced force production (Allen et al., 2001). However, caffeine and other compounds, which maximally activate RyR1 channels and cause sudden SR Ca^{2+}

release, can briefly normalize tetanic Ca^{2+} concentrations (Allen at al., 2001). Thus alterations of RyR1-dependent SR Ca^{2+} release mechanisms are likely to be involved in fatigue development.

[00131] Measurements of SR Ca^{2+} load have demonstrated a decreased pool of releasable Ca^{2+} during skeletal muscle fatigue, which may be one of the causes of reduced SR Ca^{2+} release during fatigue. (Cooke at al. 1985). Another theory involves increased intracellular inorganic phosphate concentrations ($[P_i]_i$) precipitating Ca^{2+} in the SR storage organelle (Allen, 2001;Cooke, 1985). However, $[P_i]_i$ increases occur during the early phase of fatigue resulting from rapid ATP breakdown whereas the decline of tetanic $[Ca^{2+}]_i$ occurs late in fatigue (phase iii). Moreover, after repeated, stretched contractions resting $[Ca^{2+}]_i$ was found elevated while stimulated, tetanic $[Ca^{2+}]_i$ decreased (Warren et al. 1993; Balnave et al. 1995). The increased resting $[Ca^{2+}]_i$ may initiate chronic impairment of EC coupling for example by activating proteases that can damage the SR Ca^{2+} release channel (Lamb et al., 1995 ; Chin et al., 1996; Bruton et al., 1996).

[00132] Fatigue from chronically sustained exercise may be caused by SR Ca^{2+} leak resulting from defective closure of the RyR1 channel and partial depletion of the SR Ca^{2+} store contributing to diminished force production and increased resting $[Ca^{2+}]_i$ interfering with muscle relaxation and when sustained causing muscle degeneration. Recent data shows that an evolutionary conserved stress pathway, the fight-or-flight response, specifically controls RyR1 Ca^{2+} release in skeletal muscle (Gaburjakova et al., 2001; Marx et al., 2001) and that abnormal, chronic activation of this stress pathway causes SR Ca^{2+} leak contributing to muscle fatigue (Reiken et al., 2003).

[00133] RyR1/calcium release channels become PKA hyperphosphorylated and depleted of the stabilizing protein calstabin1 during exercise. RyCal compounds of the invention increase the binding affinity of calstabin1 to PKA hyperphosphorylated RyR1. These compounds are called "calcium channel stabilizers" or "RyCals" and are in a class of 1,4-benzothiazepines and related structures. In a non-limiting example, treatment with a RyCal compound improves exercise performance of mice running on a treadmill. Furthermore, there is evidence that a calcium leak via PKA hyperphosphorylated RyR1 channels causes muscle damage due to activation of calcium-dependent proteases and RyCals prevent the calcium leak and inhibit muscle damage during chronic exercise. In certain

embodiments, RyCal compound can be used to treat, prevent or improve muscle fatigue in chronic diseases including but not limited to heart failure, AIDS, cancer, renal failure, chronic obstructive pulmonary disease, hypertension, asthma, hyperthyroidism, chronic muscle fatigue. In other embodiments, RyCal compound can be used to treat or improve muscular dystrophies. In other embodiments, treatment with RyCal compound can prevent or reduce muscle fatigue which can improve exercise performance in individuals who are exposed to sustained chronic stress and/or physical exercise. In other embodiments, treatment with RyCals can prevent or reduce muscle fatigue which can improve exercise performance in individuals who are exposed to strenuous physical exercise.

[00134] Skeletal muscles become weaker with intense use also referred to as fatigue. Moreover, repeated stretch-dependent contractures can result in additional muscle damage and degeneration. Although fatigue is recognized as an important mechanism of limited peak performance and task failure during stress, the mechanisms that promote fatigue or muscle fiber damage are incompletely characterized. Moreover, defining molecular fatigue mechanisms may enable targeted interventions that could help prevent fatigue and muscle tissue damage (Wehrens, 2005). A key physiologic mechanism that controls skeletal muscle performance is intracellular calcium (Ca^{2+}) release from specialized Ca^{2+} stores (the sarcoplasmic reticulum, SR) via ryanodine receptor (RyR1) Ca^{2+} release channels. In skeletal muscle, plasma membrane depolarization activates voltage-gated L-type Ca^{2+} channels (LTCCs; $Ca_V1.1$) which in turn activate RyR1s on the SR mediated by direct contact between both ion channels.

[00135] Opening of RyR1 channels results in bulk SR Ca^{2+} release which activates the myofilaments and muscle contraction. Also, disease forms associated with sustained activation of the sympathetic nervous system and increased plasma catecholamine levels cause maladaptive activation of intracellular stress pathways resulting in destabilization of the RyR1 channel closed state and intracellular Ca^{2+} leak (Reiken et al. 2003; Brillantes et al. 1994). SR Ca^{2+} leak via RyR1 channels was found to deplete intracellular SR calcium stores, to increase compensatory energy consumption, and to result in significant acceleration of muscle fatigue. The stress-induced muscle defect limits peak performance and contributes to pathologic forms of muscle fatigue that permanently reduce performance. Also, destabilization of the RyR1 closed state involves depletion of the stabilizing calstabin1 (FKBP12) channel subunit (Reiken et al. 2003; Brillantes et al. 1994). Experiments

demonstrate that increasing the binding affinity of calstabin to RyR rescues channel function (Wehrens, 2003).

[00136] Skeletal muscle contraction is activated by SR Ca^{2+} release via the type 1 skeletal ryanodine receptor (RyR1). Depolarization of the T-tubule membrane activates the dihydropyridine receptor voltage sensor (Cav1.1) which in turn activates RyR1 channels via a direct protein-protein interaction causing the release of SR Ca^{2+} stores. Ca^{2+} binds to troponin C allowing actin-myosin cross-bridging to occur and sarcomere shortening. Ca^{2+} release channels comprise macromolecular complexes consisting of a homotetramer of 560 kDa RyR1 subunits that form scaffolds for proteins that regulate channel function including: protein kinase A and the phosphodiesterase PDE4D3, both of which are targeted to the channel via the anchoring protein mAKAP, PP1 (targeted via spinophilin), and calstabin1 (FKBP12). (Jayaraman, Brillantes et al. 1992; Brillantes, Ondrias et al. 1994; Marx, Reiken et al. 2000; Marx, Reiken et al. 2001)

[00137] A defect in ECC that resulted in a reduction in the amplitude of SR Ca^{2+} release would impair contraction and force generation. Eberstein and Sandow proposed impaired SR Ca^{2+} release as a likely contributor to muscle fatigue (Eberstein and Sandow 1963). Reductions in the amplitude of SR Ca^{2+} release evoked during fatiguing stimulation have been reported (Allen, Lee et al. 1989; Westerblad and Allen 1991; Allen and Westerblad 2001). In addition, it has been shown that Ca^{2+} stores decline during intense and repeated contractions (Kabbara & Allen, 1999), and that the time course of recovery from fatigue parallels the time course over which prolonged depression of SR Ca^{2+} release is observed (Westerblad, Bruton et al. 2000). Furthermore, reduction of free SR Ca^{2+}, due to inorganic calcium phosphate salt precipitation during fatigue, has been proposed (Allen and Westerblad 2001).

[00138] Evidence of defective SR Ca^{2+} release in fatigued muscle prompted examination of the role of RyR1 mediated SR Ca^{2+} release in skeletal muscle fatigue. The binding of calstabin1 (FKBP12) to RyR1 stabilizes the closed state of the channel and facilitates coupled gating between neighboring channels (Brillantes, Ondrias et al. 1994; Marx, Ondrias et al. 1998). Pharmacologic depletion of calstabin1 from RyR1 (with rapamycin or FK506 both of which bind to calstabin1 and dissociate it from the RyR1 macromolecular complex) promotes subconductance states and, in intact skeletal muscle,

can cause a rapid loss of depolarization-induced contraction (Lamb and Stephenson 1996). Mutation of RyR1 resulting in the loss of calstabin1 binding causes impaired ECC with reduced maximal voltage-gated SR Ca^{2+} release without affecting the SR Ca^{2+} store content (Avila, Lee et al. 2003). Genetic deletion of *FKBP12 (calstabin1)* induced no gross histological or developmental defect in skeletal muscle, though severe developmental cardiac defects were observed which precluded detailed assessment of skeletal muscle function (Shou, Aghdasi et al. 1998). Whereas skeletal muscle-specific knock-out of *FKBP12 (calstabin1)* resulted in reduced voltage-gated SR Ca^{2+} release and increased L-type channel currents in isolated myotubes (Tang, Ingalls et al. 2004). In extensor digitalis longus (EDL), but not soleus or diaphragm, reduced maximal tetanic force and a rightward shift in force-frequency relationships were observed (Tang, Ingalls et al. 2004). These data indicated that calstabin1 modulates the gain of ECC in skeletal muscle.

[00139] The binding of calstabin1 to RyR1 is regulated by PKA phosphorylation at RyR1-S2843 (position S2844 in the mouse RyR1 sequence) (Reiken, Lacampagne et al. 2003). Phosphorylation at RyR1-S2843 increases the mean open probability of RyR1 in the lipid bilayer (Reiken, Lacampagne et al. 2003). RyR1-S2843A mutant channels could not be PKA phosphorylated and did not show the same PKA-dependent increase in open probability. An RyR1-S2843D mutation mimicked PKA phosphorylation with an increased open probability and destabilized open and closed states (Reiken, Lacampagne et al. 2003). The role of PKA phosphorylation of RyR1 is still under investigation as other groups have found little or no effect on channel function (Stange, Xu et al. 2003). Other post-translational modifications of RyR1 which might modulate calstabin1 binding to RyR1 have been suggested, including oxidation, and glutathionylation of the up to 50 free (reduced) thiols on each RyR monomer.

[00140] SR Ca^{2+} leak has been documented as aberrant calcium sparks in myofibers following intense exercise and in a model of muscular dystrophy (Wang, Weisleder et al. 2005), possibly due to defective RyR1 function. Chronic activation of the sympathetic nervous system (SNS) during heart failure is associated with early skeletal muscle fatigue and PKA hyperphosphorylation of RyR1 at Ser2844 (meaning that on average 3-4 of the four PKA sites in each homotetrameric channel are PKA phosphorylated in heart failure skeletal muscle), calstabin1 depletion from the RyR1 complex, and a gain-of-function

channel defect (Reiken, Lacampagne et al. 2003). RyR1 dysfunction in skeletal muscle leads to altered local subcellular Ca^{2+} release events (Ward, Reiken et al. 2003).

[00141] Modifications in the RyR1 complex could alter and are likely to limit peak muscle performance, increase muscle fatigue, and contribute to muscle damage during prolonged or high intensity exercise. In certain aspects the invention provides use of a mouse model of chronic, high-intensity, forced exercise to assess the role of the RyR1 channel in skeletal muscle fatigue. As described herein, the RyR1 channel macromolecular complex undergoes remodeling during exercise such that it is progressively PKA hyperphosphorylated, and depleted of PDE4D3 and calstabin1. Functionally, this remodeling is associated with "leaky" channels (increased open probability) and activation of the calcium-activated protease calpain, and leakage of creatine kinase (CPK) into the plasma, which is consistent with muscle damage. These changes are further associated with decreased force production in isolated muscles and impaired exercise capacity and are exacerbated in mice with PDE4D deficiency or muscle specific calstabin1 deficiency. Preventing the RyR1 channel leak with a calcium channel stabilizer S107, which enhances binding of calstabin1 to RyR1, inhibits calpain activation, CPK leak and improves exercise performance. Therefore, remodeling of the RyR1 channel complex that causes leaky channels, activation of the calcium-activated protease calpain, and leakage of creatine kinase (CPK) into the plasma, is a mechanism involved in muscle fatigue during chronic or high intensity exercise.

[00142] Confocal imaging studies of intracellular Ca^{2+} release (Ca^{2+} sparks) in muscle cells after mild and strenuous treadmill exercise, showing that abnormal Ca^{2+} spark activity is induced by fatiguing exercise (Wang et al. 2005). Moreover, myofibers with abnormal Ca^{2+} spark activity resulting form strenuous exercise show histological signs of degeneration from toxic Ca^{2+} effects also known as 'dystrophic remodeling' (Wang et al. 2005). It is likely that sustained exercise over weeks and months results in RyR1 dysfunction, intracellular Ca^{2+} leak, depressed muscle performance, and dystrophic muscle remodeling. Furthermore, 1,4-benzothiazepine based drugs enhance peak muscle performance and prevent dystrophic remodeling by fixing stress-induced intracellular Ca^{2+} leak.

[00143] Animal models can establish, *in vivo* and at the level of isolated skeletal muscle cell, and single RyR1 channel, that defects in RyR1 function cause muscle fatigue and

dystrophic remodeling. Use of these animal models of fatigue and the characterized muscle, cells, and channel techniques allows tests of therapeutic approaches based on fixing the leak in RyR1 which will result in improved skeletal muscle performance, decreased muscle fatigue, and reduced dystrophic remodeling during chronic forms of exercise.

[00144] **Muscular Dystrophy**

[00145] Myotonic dystrophy type 1 (DM1), the most common muscular dystrophy in adults (1 in 7,400 live births), is a multisystemic disorder caused by a CTG trinucleotide repeat expansion in the 3' untranslated region of the myotonic dystrophy protein kinase (DMPK) gene which causes progressive muscle weakness, inherited muscle hyperexitability (myotonia), cardiac conduction defect, cataract, and insulin resistance. (Bachinski LL, Udd B, Meola G, et al. Confirmation of the type 2 myotonic dystrophy (CCTG)n expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. *Am J Hum Genet.* Oct 2003;73(4):835-848. Hamshere MG, Harley H, Harper P, et al. Myotonic dystrophy: the correlation of (CTG) repeat length in leucocytes with age at onset is significant only for patients with small expansions. *J Med Genet.* Jan 1999;36(1):59-61; Liquori CL, Ricker K, Moseley ML, et al. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science.* Aug 3 2001;293(5531):864-867.) The mutant DMPK messenger RNA (mRNA) containing an expanded CUG repeat is retained in the nucleus and protein levels are reduced. (Mankodi A, Logigian E, Callahan L, et al. Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science.* Sep 8 2000;289(5485):1769-1773) The RNA repeat expansion changes the chromatin structure, silences the expression of the flanking *SIX5* gene which codes for a transcription factors, and disrupts regulation of gene expression during development and exercise. (Ebralidze A, Wang Y, Petkova V, et al. RNA leaching of transcription factors disrupts transcription in myotonic dystrophy. *Science.* Jan 16 2004;303(5656):383-387)

[00146] The cause of the most severe symptoms including muscle weakness and progressive muscle wasting appear to be caused by elevated intracellular Ca^{2+} concentrations and subsequent myofiber degeneration in DM1. (Jacobs AE, Benders AA, Oosterhof A, et al. The calcium homeostasis and the membrane potential of cultured muscle cells from patients with myotonic dystrophy. *Biochim Biophys Acta.* Nov 14 1990;1096(1):14-19) Moreover, a

recent study has linked disturbed Ca^{2+} cycling in DM1 to aberrant splicing of RyR1 and SR Ca^{2+} ATPase (SERCA1) mRNAs. (Kimura T, Nakamori M, Lueck JD, et al. Altered mRNA Splicing of the Skeletal Muscle Ryanodine Receptor and Sarcoplasmic/Endoplasmic Reticulum Ca2+-ATPase in Myotonic Dystrophy Type 1. *Hum Mol Genet.* Jun 22 2005.) A muscle-specific genetic mouse model HSA^{LR} of DM1 exists in which expanded CUG repeat expression results in a DM-like phenotype. (Mankodi A, Logigian E, Callahan L, et al. Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science.* Sep 8 2000;289(5485):1769-1773) HSA^{LR} have a myotonic phenotype in the absence of muscle fiber necrosis and the short- versus long-repeat expressing mouse lines show relatively less or more histopathological signs of muscle regeneration and repair, respectively. (Mankodi A, Logigian E, Callahan L, et al. Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. *Science.* Sep 8 2000;289(5485):1769-1773) Since HSA^{LR} mice with short- or long repeat expression show no signs of muscle weakness and since RyR1 alterations have been linked to DM1, the HSA^{LR} mice provide a model to study the effects of exercise and activation of the sympathetic nervous sytem in these mice. After characterizing RyR1 channel composition, phosphorylation status, and function, the HSA^{LR} mice can be challenge with sustained exercise tests and treated with RyCal compounds. Since the mechanism by which transcripts with expanded CUG repeats cause myotonia and muscle degeneration in DM1 is not known, this would provide 1) study of a genetic animal model of severe fatigue, 2) elucidation of the key molecular mechanism of DM1, and 3) developing a therapeutic rationale for the most common muscular dystrophy in adults.

[00147] **Stress pathways and muscle fatigue**

[00148] Sustained activation of intracellular stress pathways such as occurring during strenuous physical exercise, for example but not limited to combat, can result in reduced muscle performance and tissue damage. A major determinant of muscle damage may occur due to toxicity of continuously high catecholamine levels resulting in intracellular Ca^{2+} leak. This concept is supported by: 1) physiologic (non-combat) exercise or stress was reported to induce muscle weakness, cramps, and tissue atrophy in susceptible individuals (stress-induced rhabdomyolysis) (Wappler et al., 2001); 2) strenuous but not mild treadmill exercise induces a significantly elevated Ca^{2+} spark frequency in muscle cells indicating intracellular Ca^{2+} leak (Wang et al., 2005); 3) excess catecholamine levels were found in malignant hyperthermia (MH) and central core disease (CCD) contributing to uncontrolled intracellular

Ca^{2+} release (Monnier et al. 2000; MacLennan et al. 1995); 4) a majority of MH/CCD were linked to RyR1 missense mutations (Loke et al., 2003); 5) a hyperadrenergic state as occuring in heart failure causes RyR1 hyperphosphorylation, Ca^{2+} leak, and skeletal muscle fatigue (Reiken et al. 2003); and 6) excess plasma catecholamine levels by activating β-adrenergic receptors, intracellular cAMP synthesis and protein kinase A phosphorylation results in muscle damage (Goldspink et al., 2004; Tan et al. 2003). Stress-dependent muscle damage and dysfunction occurs at the interface between intracellular catecholamine effectors (protein kinase A, PKA) and intracellular Ca^{2+} release. Skeletal ryanodine receptor (RyR1) Ca^{2+} release channels constitute intracellular scaffolds that integrate PKA-mediated stress signaling and regulation of intracellular Ca^{2+} release and therefore determine the gain of EC coupling and muscle function. Importantly, chronically increased PKA phosphorylation of RyR1 occurring from a chronic hyperadrenergic state *in vivo* depletes the stabilizing calstabin1 subunits resulting in SR Ca^{2+} leak and muscle fatigue. Moreover, these observations extend to an *in vivo* animal model of fatigue. Therefore, sustained activation of the sympathetic nervous system during continued muscle performance contributes to increased fatigue development and dystrophic skeletal muscle damage. Since skeletal RyR1 Ca^{2+} release channel is PKA hyperphosphorylated and depleted of the stabilizing calstabin1 subunit after 21 days of intense exercise, wherein similar alterations result in intracellular Ca^{2+} leak in animals with heart failure, it is likely that chronic (> 1 week) forms of exercise result in adverse intracellular Ca^{2+} leak from defective RyR1 channels.

[00149] In one aspect, the invention establishes molecular mechanisms of muscle fatigue occurring from chronically sustained muscle performance. In another aspect the invention provides methods for treating or preventing detrimental intracellular Ca^{2+} leak and muscle damage by administering novel 1,4-benzothiazepine derivatives. The skeletal ryanodine receptor (RyR1) channel is comprised of 4 RyR1 subunits and associated proteins that bind to the cytoplasmic domain of the channel forming a macromolecular signaling complex. Certain aspects of the invention examine mechanisms by which allosteric modulators regulate RyR1 function. Two specific forms of allosteric modulation are examined: 1) regulation of the channel by cAMP-dependent protein kinase A (PKA) that potently activates channel gating; 2) depletion of the stabilizing subunit calstabin1 from the RyR1 channel during chronically increased PKA phosphorylation resulting from chronic activation of the sympathetic nervous system by strenuous, sustained exercise.

[00150] Dysregulation of RyR1 by PKA during sustained exercise causes intracellular Ca^{2+} leak and may be a mechanism of increased muscle fatigue and dystrophic remodeling. In certain aspects, the invention determines the effects of fatiguing exercise on 1) RyR1 PKA phosphorylation; 2) RyR1 channel function examined using RyR1 channels reconstituted into planar lipid bilayers; 3) Calstabin1 binding to RyR1; 4) intracellular Ca^{2+} sparks in isolated myofibers; 5) isolated skeletal muscle function; 6) mitochondrial integrity, 7) *in vivo* exercise performance, 8) skeletal muscle histology, fiber type composition and oxidative capacity, 9) creatine kinase (CK) plasma levels, 10) RyR1 PKA phosphorylation and calstabin1 depletion in leukocytes. Calstabin1 depletion during chronically sustained exercise and PKA hyperphosphorylation causes RyR1 hyperactivity, intracellular Ca^{2+} leak, depletion of SR Ca^{2+} stores, accelerated fatigue and dystrophic muscle remodeling.

[00151] In certain embodiments, treatment with any RyCal compound normalizes RyR dysfunction and intracellular Ca^{2+} leak. RyR1, which are protein kinase A (PKA) hyperphosphorylated and "leaky" in heart failure models and during strenuous exercise, can rebind calstabin1 after RyCal compound treatment, which normalizes single channel and improves muscle performance in heart failure. It is likely that RyCals *in vivo* and in cells treated with RyCals prevent intracellular Ca^{2+} leak and normalize RyR1 channel function during sustained exercise.

[00152] By preventing RyR1 Ca^{2+} leak, RyCal compounds improves skeletal muscle fatigue and dystrophic remodeling during strenuous exercise as occurs in combat. By using two animal models of muscle fatigue (swimming and running on a treadmill) in mice and rats, certain aspects of the invention show that treatment with RyCal compounds prevents depletion of calstabin1 from RyR1, which decreases muscle fatigue, improves performance, and inhibits dystrophic muscle remodeling.

[00153] Certain aspects of the invention identify molecular mechanisms of muscle fatigue in myotonic dystrophy (DM1). In certain embodiments, by preventing RyR1 Ca^{2+} leak, RyCal compounds improves skeletal muscle fatigue times and dystrophic remodeling in mouse models of myotonic dystrophy.

[00154] Other aspects of the invention characterize molecular fatigue mechanisms in genetic mouse models. In certain embodiments, calstabin1 and PDE4D3 knockout mice can be used to further explore molecular fatigue mechanisms and dysregulation during sustained

exercise states that contribute to intracellular Ca^{2+} leak. In other aspects, the compounds of the invention reduce calpain activity. In other aspects, the compounds of the invention reduce plasma creatine kinase activity.

[00155] Other aspects of the invention, characterize mechanisms that destabilize the closed state of intracellular calcium (Ca^{2+}) release channels during chronic activation of the sympathetic nervous system as occurs during sustained exercise and/or in combat. There are two allosteric modulators of skeletal ryanodine receptors (RyR1s), one is PKA and the other one is a stabilizing protein subunit of the channel (calstabin1) (Wehrens et al., 2004). Other aspects of the invention disclose therapeutic and preventive measures wherein a drug molecule that rebinds calstabin1 may prevent skeletal muscle fatigue by normalizing the skeletal (RyR1) ryanodine receptor gating. Protein kinase A (PKA) hyperphosphorylation of RyR1 during chronic activation of the sympathetic nervous system was shown to result in SR Ca^{2+} leak as a cause of fatigue (Reiken et al., 2003) which was reversed by JTV519 (Wehrens et al., 2005). In certain aspects, the invention provides, thorough characterization of the effects of strenuous exercise on RyR1 Ca^{2+} leak, *in vivo* and *ex vivo* muscle performance and energetic metabolism, methods for the use of RyCal compounds to overcome muscle fatigue and to prevent muscle damage and/or fatigue during strenuous, and/or sustained exercise, and muscle damage and/or fatigue associated with defective skeletal muscle function, or any disease condition.

[00156] Ceratin aspects of the invention characterize mechanisms that destabilize the closed state of intracellular calcium (Ca^{2+}) release channels which is a prerequisite for muscle relaxation to occur and prevents damage of myofibers from uncontrolled intracellular SR Ca^{2+} leak. Chronic activation of the sympathetic nervous system during forced and sustained exercise caused RyR1 PKA hyperphosphorylation, calstabin1 depletion, and a defective channel closed state are consistent with SR Ca^{2+} leak. Since stress and physical performance in combat are considered significantly more severe compared to the animal use protocols, it can be extrapolated that muscle fatigue and dystrophic degeneration from RyR1 Ca^{2+} leak represents a more severe phenotype in warfighters. The focus is on two allosteric modulators of skeletal ryanodiné receptors (RyR1s), one PKA as a key stress pathway and the other one a stabilizing protein subunit of the channel (calstabin1).

[00157] Ceratin aspects of the invention address potential therapeutic and preventive measures in that a drug molecule rebinding calstabin1 may prevent skeletal muscle fatigue by normalizing the skeletal (RyR1) ryanodine receptor channel gating. Protein kinase A (PKA) hyperphosphorylation of RyR1 during chronic activation of the sympathetic nervous system was shown to result in SR Ca^{2+} leak as a cause of fatigue. (Wehrens XH, Lehnart SE, Reiken S, et al. Enhancing calstabin binding to ryanodine receptors improves cardiac and skeletal muscle function in heart failure. *Proc Natl Acad Sci U S A.* Jul 5 2005;102(27):9607-9612; Ward CW, Reiken S, Marks AR, et al. Defects in ryanodine receptor calcium release in skeletal muscle from post-myocardial infarct rats. *Faseb J.* Aug 2003;17(11):1517-1519.) Certain aspects of the invention are directed to thorough characterization of the effects of strenuous exercise on RyR1 Ca^{2+} leak, *in vivo* and *ex vivo* muscle performance and energetic metabolism, and the use of RyCal compounds to improve muscle fatigue times, to increase exercise capacity, and to prevent muscle damage during and/or after strenuous, sustained exercise.

[00158] Life quality and prognosis in heart failure patients is decreased due to skeletal muscle dysfunction (e.g., shortness of breath due to diaphragmatic weakness, and exercise intolerance due to limb skeletal muscle fatigue) (Harrington et al. 1997). Recent studies have identified dysregulation of intracellular Ca^{2+} release from the SR as a pathogenic mechanism underlying skeletal muscle dysfunction in heart failure (Reiken S, Lacampagne A, Zhou H, et al. PKA phosphorylation activates the calcium release channel (ryanodine receptor) in skeletal muscle: defective regulation in heart failure. *J Cell Biol.* Mar 17 2003;160(6):919-928.;Ward, 2003; Perreault CL, Gonzalez-Serratos H, Litwin SE, et al. Alterations in contractility and intracellular Ca2+ transients in isolated bundles of skeletal muscle fibers from rats with chronic heart failure. *Circ Res.* Aug 1993;73(2):405-412.) Heart failure in animals with myocardial infarcts causes significantly accelerated fatigue which is intrinsic to skeletal muscle as measured by the time for the tetanic force to fall below 50% of the maximal contraction (Reiken, 2003).

[00159] **Skeletal RyR1 are PKA hyperphosphorylated and depleted of calstabin1**

[00160] Previous studies have suggested that skeletal muscle RyR1 channels are PKA hyperphosphorylated and depleted of calstabin1 in a pacing-induced canine model of heart failure and a rat post-myocardial infarct model (Reiken et al., 2003; Ward et al., 2003).

Furthermore, in a mouse model of post-myocardial infarction heart failure, RyR1 in soleus muscle is PKA-hyperphosphorylated. In certain embodiments, treatment with a RyCal compound, allows rebinding of calstabin1 to RyR1 despite intense chronic exercise.

[00161] Beta-adrenergic stimulation increases the gain of the EC coupling when enhanced muscle performance is required during exercise or stress (fight-or-flight response). Binding of catecholamines to β-adrenoceptors activates a G-protein coupled intracellular signaling cascade, which leads to increased intracellular cAMP concentrations and activation of protein kinase A (PKA). PKA is targeted to RyR1 via mAKAP forming a signaling complex with the skeletal Ca^{2+} release channel (Reiken et al. 2003). RyR1 phosphorylation by PKA increases the channel open probability and SR Ca^{2+} release (Reiken et al., 2003; Wehrens et al., 2004).

[00162] Data from skeletal myofibers have confirmed intracellular Ca^{2+} leak from enhanced RyR1 activity after strenuous exercise consistent with an increased maximal rate of SR Ca^{2+} release. PKA-hyperphosphorylation of RyR1 results in depletion of calstabin1 (FKBP12) from the channel complex due to a reduced binding affinity for calstabin1. Chronic depletion of calstabin1 from the RyR1 channel complex relieves an intrinsic inhibition of the channel and induces uncontrolled intracellular Ca^{2+} leak and reduced fatigue resistance during a sustained hyperadrenergic state. Skeletal muscle fatigue is increased in heart failure patients and in animal models of heart failure (Reiken et al., 2003; Harrington et al., 1997; Perreault et al., 1993; Lunde et al. 2001; Lunde et al. 1998). In both patients and animals with heart failure the skeletal RyR1 channel isoform was found PKA hyperphosphorylated and depleted of the stabilizing calstabin1 subunits (Reiken et al., 2003; Wehrens et al., 2004). Increased fatigue and RyR1 hyperphosphorylation are associated with an increased Ca^{2+} spark frequency and a decreased Ca^{2+} spark amplitude in skeletal myofibers in heart failure animals consistent with intracellular Ca^{2+} leak and decreased SR Ca^{2+} concentrations (Reiken et al., 2003). Therefore, most likely increased muscle fatigue results from a chronic hyperadrenergic state causing an intracellular Ca^{2+} leak via defective RyR1 channels (Reiken et al., 2003).

[00163] However, it is important to conceptualize that the role of external Ca^{2+} ions in mammalian skeletal muscle contraction is not completely understood. The LTCC and RyR isoforms in skeletal and cardiac muscles are different, with skeletal muscle expressing the

LTCC $\alpha 1_S$ subunit (Tanabe et al., 1988) and RyR1, (Marks et al., 1989) and cardiac muscle expressing the LTCC $\alpha 1_C$ subunit (Mikami et al., 1989) and RyR2 (Nakai et al., 1990). RyR1 in skeletal muscle does not depend on Ca^{2+} influx via LTCC $\alpha 1_S$ to activate SR Ca^{2+} release as evidenced by continuous EC coupling in skeletal muscle cells when external Ca^{2+} is removed or when Ca^{2+} channel blockers are present (Armstrong et al., 1972; Dulhunty et al., 1988 ; Gonzalez-Serratos et al., 1982). Later experimental findings support RyR1 activation by physical coupling with LTCC $\alpha 1_S$ (Rios et al., 1987; Tanabe et al., 1990). RyR1 Ca^{2+} leak likely increases the cellular energy demands by compensatory SR Ca^{2+} ATPase uptake consuming more ATP which may contribute to earlier skeletal muscle fatigue. From direct oxygen measurements on the surface of contracting muscle preparations, it is estimated that total ATP consumption by SR Ca^{2+} ATPases is significantly elevated in heart failure (Meyer et al., 1998) likely resulting from intracellular Ca^{2+} leak. In agreement with decreased SR Ca^{2+} concentrations due to RyR1 Ca^{2+} leak, muscle-specific calstabin1 knockout increases LTCC Ca^{2+} influx and reduces maximal voltage-gated intracellular Ca^{2+} release (Tang et al., 2004).

[00164] Recent studies have demonstrated defective function of RyR1 channels in skeletal muscle during heart failure, which were analogous to those found in RyR2 channels in failing myocardium: PKA hyperphosphorylation of RyR1 and depletion of calstabin1 (Marx et al., 2000; Reiken et al., 2003; Wehrens et al., 2005). These findings suggest that defects in RyR1 function alter intracellular Ca^{2+} handling, thereby contributing to early fatigue in skeletal muscles. Depletion of calstabin1 from the RyR1 macromolecular complex may also uncouple channels from one another and allow stochastic as opposed to coupled gating (Marx et al., 1998), thus providing an attractive hypothesis for explaining the altered Ca^{2+} spark behaviour in skeletal muscle with reduced fatigue resistance (Ward et al., 2003). Thus, alterations in RyR1 could play a significant role in the skeletal muscle specific force decrements and reduced exercise-tolerance seen in models of increased muscle fatigue.

[00165] **Methods**

[00166] Aerobic exercise can be defined as a form of physical exercise that increases the heart rate and enhances oxygen intake to improve performance. Examples of aerobic exercise are running, cycling, and swimming. In certain embodiments, mice were challenged by aerobic exercise (forced swimming) for 90 mins twice daily. The animals were

accustomed to swimming in preliminary training sessions: day -3 twice 30 mins, day -2 twice 45 mins, day -1 twice 60 mins, day 0 and following twice 90 mins. Mice were then exercised for 1, 7, or 21 additional, consecutive days for 90 mins twice daily. Between swimming sessions separated by a 4 hour rest period the mice are kept warm and given food and water. An adjustable-current water pool was used to exercise mice by swimming. An acrylic pool (90 cm long x 45 cm wide x 45 cm deep) was filled with water to a depth of 25 cm. A current in the pool was generated with a pump. The current speed during the swimming session was at a constant speed of 1 l/min flow rate. The water temperature was maintained at 34°C with an electric heater. Age- and weight-matched mice are used to exclude differences in buoyancy from body fat.

[00167] Using forced swimming as an efficient protocol to increase skeletal muscle aerobic capacity in mice (Evangelista et al., 2003), the composition and phosphorylation status of the skeletal RyR1 channel complex was investigated. Unexpectedly, after 3 weeks of 90 mins swimming twice daily, C57Bl6 wild-type mice showed significantly increased RyR2 phosphorylation by PKA while CaMKII phosphorylation was not changed. RyR1 protein expression was stable, however, RyR1 channels were depleted of the stabilizing subunit calstabin1 (FKBP12). RyR1 hyperphosphorylation and calstabin1 depletion are consistent with leaky RyR1 channels that cause intracellular Ca^{2+} leak.

[00168] To investigate the influence of the duration of sustained exercise on the RyR1 Ca^{2+} release channel defect, mice were exposed to swimming for 1, 7, or 21 days followed by immediate sacrifice. Longer exposure to sustained exercise shows a significant increase of RyR1 PKA hyperphosphorylation beginning at 7 days and saturating at 21 days.

[00169] Moreover, a mouse model of muscular dystrophy which is known to result in intracellular Ca^{2+} leak and dystrophic muscle changes was investigated. Surprisingly, soleus muscles of the dystrophin-deficient *mdx* mouse show calstabin1 depletion in the absence of increased RyR1-Ser2844 phosphorylation. These results identify RyR1-Ser2844 PKA hyperphosphorylation as a specific event in exercise-induced RyR1 dysfunction.

[00170] Additionally, the histological changes in the fast-twitch muscles of mice exposed to 3 weeks of exercise by swimming were characterized. Cross-sections of the mouse *M. extensor digitorum longus* (EDL) showed histological changes consistent with myofiber degeneration from intracellular Ca^{2+} overload from defective RyR1 channel.

Therefore sustained exercise for 90 mins twice daily triggers a distrophic phenotype in EDL muscles of normal C57Bl6 mice.

[00171] Up-regulation of intracellular SR Ca^{2+} release by cAMP-dependent signaling pathways augments the gain of excitation-contraction coupling during peak muscle performance. (Reiken, 2003) Therefore transient PKA phosphorylation of skeletal RyR1 Ca^{2+} release channels represents a key mechanism of the fight-or-flight response. Studies have established that dysregulation of RyR1 Ca^{2+} release channels occurs during strenuous fatiguing exercise. Mice which underwent rigorous exercise for 3 weeks showed significantly increased levels of RyR1 PKA phosphorylation, increased RyR1 channel activity, and dystrophic histological changes. Chronic RyR1 hyperphosphorylation results in depletion of the stabilizing calstabin1, functional channel defects, suggesting intracellular Ca^{2+} leak. At the level of the muscle cell, this model was recently confirmed by a report that increased Ca^{2+} spark frequency after fatiguing exercise in mouse skeletal muscle (Wang et al., 2005). Moreover, it was shown that intracellular Ca^{2+} leak from chronic exercise acts as dystrophic signal in mammalian skeletal muscle (Wang, 2005). This confirms previous observations in cardiac muscle, where hormonal and structural changes contribute to intracellular Ca^{2+} leak causing defective excitation-contraction coupling (Gomez, 1997). From these studies (Wehrens et al., 2005; Reiken et al., 2003) and the Ca^{2+} spark data in fatiguing muscle (Wang et al., 2005) it is likely that intracellular Ca^{2+} leak represents a key pathology that accelerates muscle fatigue and causes dystrophic remodeling of muscles. Accordingly, extended investigation of fatigue models at the *in vivo*, isolated muscle, muscle cell, mitochondria, and single RyR1 channel level will characterize detrimental effects during peak performance on muscle fatigue and dystrophic remodeling.

[00172] 1,4-benzothiazepine derivatives prevent muscle dysfunction from intracellular Ca^{2+} leak under conditions of sustained sympathetic nervous system activation occurring from heart failure (Wehrens et al., 2005). In certain aspects, the invention provides RyCal compounds and their use to enhance skeletal muscle performance and to reduce muscle dysfunction during sustained stress as occurs under combat conditions. Defects in the RyR1 Ca^{2+} release channel due to dysregulation by PKA allow rationalizing of a therapeutic concept: intracellular SR Ca^{2+} leak and muscle dysfunction can be prevented by a drug that increases binding of the stabilizing calstabin1 subunit to the RyR1 channel complex and thereby inhibits SR Ca^{2+} leak and dystrophic muscle remodeling from sustained stress or

exercise. Applying a drug to prevent SR Ca^{2+} leak can therefore enhance a variety of important physiologic skills which are prone to stress-induced dysfunction. In certain aspects, the invention provides that enhanced binding of calstabin1 to RyR1 by RyCal compounds prevents muscle fatigue. Using animal models of physiologic (swimming and running on a treadmill) and/or pharmacologic activation (β-adrenergic receptor agonists) of the sympathetic nervous system combined with different degrees of exercise exposure, it can be tested whether RyCal compounds improve skeletal muscle function and prevent dystrophic muscle degeneration. This may lead to a pharmacologic approach based on allosteric modulation of RyR1 that can result in improved human performance during sustained stress and prevent adverse tissue damage thereby shortening recovery times. In other aspects, the invention provides pharmacologic approaches based on allosteric modulation of RyR1 that can result in improved human performance during sustained stress and prevent adverse tissue damage and shorten recovery times.

[00173] RyR1 PKA phosphorylation: quantification of skeletal RyR1 channel phosphorylation by protein kinase A (PKA) following sustained exercise for 2 days, 1 week, and 3 weeks using two independent techniques (RyR1 PKA phospho-epitope detection by specific antibody; incorporation of radiolabeled phosphate by backphosphorylation essay).

[00174] RyR1 CaMKII phosphorylation: quantification of skeletal RyR1 channel phosphorylation by Ca^{2+}-calmodulin protein kinase II (CaMKII) following sustained exercise for 2 days, 1 week, and 3 weeks using RyR1 CaMKII phospho-epitope detection by specific antibody essay developed in our laboratory. This will allow characterizing specific defects resulting from chronic PKA phosphorylation and/or secondary activation of CaMKII signaling by intracellular Ca^{2+} leak mechanisms.

[00175] Calstabin1 depletion in the RyR1 complex: quantification of depletion of the channel stabilizing subunit calstabin1 (FKBP12) from the RyR1 channel complex by immunoprecipitation techniques following sustained exercise for 2 days, 1 week, and 3 weeks. Calstabin1 depletion occurs from Ser2843 phosphorylation by PKA.

[00176] RyR1 functional defects --*in vivo* development of "leaky" RyR1 channels: electrophysiologic characterization of RyR1 single-channel activity and open probability following sustained exercise for 2 days, 1 week, and 3 weeks. This allows for a comprehensive and sensitive assessment of SR Ca^{2+} release channel defects and leak

mechanisms that are known to contribute to muscle fatigue. Moreover, these data will allow developing a rationale for preventive treatment of RyR1 Ca^{2+} leak using a small lead RyCal compounds.

[00177] Improvement of fatigue from sustained exercise: quantification of *in vivo* fatigue using two independent exercise performance tests: swimming and running on a treadmill. The treadmill test will be combined with electrocardiogram telemetry in a subgroup of animals to allow for objective correlation of increased heart rates during exercise with fatigue symptoms. Moreover, plasma and muscle catecholamine levels will be determined to verify sustained activation of the sympathetic nervous system. Three weeks of maximal fatiguing swimming exercise induces progressive RyR1 dysfunction and dystrophic skeletal muscle changes during a low flow rate of 1 l/min (baseline condition which prevents mice from floating passively). To objectively quantify fatigue times during swimming exercise, video tracking system can be used (San Diego Instruments Incorporated) which automatically tracks and digitizes spatio-temporal movements of 8 mice. Improvement of fatigue will be assessed by time to fatigue (defined as significant increase in 2D distance or activity over time).

[00178] Improvement of maximal exercise capacity: To determine maximal endurance exercise capacity (i.e., time to exhaustion), the maximum swim times are measured at a flow rate of 7 l/min in repeated measurements three times a week. To reduce the inherent variation in swimming capacity, mice whose mean maximum swim times vary by more than 40% than the average swim time will be excluded. A mouse is qualified as fatigued when it fails to rise to the water surface to breathe and will be rescued at this point. To verify the systemic exhaustion after swimming, plasma lactate and pH will be determined in heparinized arterial blood samples. To objectively quantify fatigue times during forced swimming exercise, we will use a video tracking system (San Diego Instruments Incorporated) which automatically tracks and digitizes spatio-temporal movements of 8 mice in a 2D plane.

[00179] Isolated skeletal muscle function: *ex vivo* characterization of intrinsic muscle resistance to fatigue stimulation or single-twitch contraction protocols following sustained exercise with placebo or a RyCal compound treatment for 2 days, 1 week, and 3 weeks. Two different forms of isolated skeletal muscles will be tested: *extensor digitorum longus* for a fast-twitch muscle and *soleus muscle* for a slow-twitch muscle. This test can determine the

effect of RyCal compounds on skeletal muscle under fatiguing exercise, or disease conditions. Skeletal muscle contraction and relaxation is critically dependent on intracellular Ca^{2+} metabolism and RyR1 function and therefore a highly sensitive test.

[00180] Intracellular Ca^{2+} leak and SR Ca^{2+} content in isolated skeletal myofibers: assessment of isolated skeletal muscle myofibers loaded with fluo-4 Ca^{2+} indicators for resting SR Ca^{2+} leak using intracellular Ca^{2+} sparks. SR Ca^{2+} content will be assessed by caffeine pulse protocols which result in complete release of the free SR Ca^{2+} pool. Previous studies have documented calcium leak resulting from a chronic hyperadrenergic state in myofibers of rats with heart failure (Reiken 2003; Ward 2003; Gomez 2001; Cheng 1996)

[00181] Changes in mitochondrial integrity from SR Ca^{2+} leak: mitochondria in mouse skeletal muscle take up Ca^{2+} which under conditions of strong physiologic muscle stimulation result in continuously elevated mitochondrial Ca^{2+} levels which stimulates mitochondrial metabolism (Rudolf et al., 2004). However, myotubes from dystrophic *mdx* mice showed significantly elevated Ca^{2+} uptake. (Robert V, Massimino ML, Tosello V, et al. Alteration in calcium handling at the subcellular level in mdx myotubes. *J Biol Chem.* Feb 16 2001;276(7):4647-4651.) Cytosolic Ca^{2+} overload is a highly toxic event that represents a common final pathway of cell death. Mitochondria are key players in cell death, and the spatial proximity of RyR1 Ca^{2+} release and mitochondrial Ca^{2+} uptake suggest, that SR Ca^{2+} leak during strenuous exercise can cause mitochondrial Ca^{2+} overload which impacts on mitochondrial structure and function and may trigger cell death. Caspase-12 is localized in the SR, is regulated by Ca^{2+}, and participates in the SR stress-induced apoptosis pathway (Yoneda et al., 2001). In certain aspects the invention characterizes the effects of fatiguing exercise on mitochondrial membrane potential by rhodamine 123 uptake, mitochondrial swelling indicating mitochondrial permeability transition by 520 nm spectrophotometry and light scatter, the amount of intra-mitochondrial Ca^{2+} by organelle incubation with $^{45}Ca^{2+}$ followed by radioactivity quantification with a liquid scintillation counter, measurement of cytochrome c from release mitochondria coactivating caspases, and staining of muscle preparations for TUNEL-positive cell nuclei. The *in vivo* effects of RyCal compounds treatment on mitochondrial integrity and function will be assessed.

[00182] Activation of intracellular proteases fragments RyR1: A direct link exists between the cytosolic Ca^{2+} elevations and the proteolysis of intracellular targets through the

activation of Ca^{2+}-dependent proteases, including calpains and caspases. Calpain activation is part of the apoptosis machinery. Increased activation of the ubiquitous calpains has been found in the mouse model of Duchenne muscular dystrophy (DMD), but null mutations of muscle specific calpain causes limb girdle muscular dystrophy 2A (LGMD2A) (Tidball et al., 2000). These findings indicate that dysregulation of calpain activity contributes to progression of muscle disease by disrupting normal regulatory mechanisms and by a generalized, nonspecific increase of proteolytic capacity. RyR1 and other components of the Ca^{2+} cycling machinery are targets of and cleaved by caspases and calpains (Johnson et al., 2004; Shevchenko at al., 1998). We will therefore investigate if Ca^{2+} dependent protease and/or caspase activation result in RyR1 cleavage following strenuous exercise and if JTV519 by inhibiting SR Ca^{2+} leak can prevent activation of unspecific proteolysis.

[00183] Histologic changes of skeletal muscle from strenuous, fatiguing exercise: Dystrophic changes from sustained exercise may result in muscle fiber necrosis and progressive muscle wasting and weakness. Histological analysis of skeletal muscles will include analysis for eosinophilic hypercontracted muscle fibers, necrotic fibers, ongoing muscle regeneration, and the proliferation of fibroblasts within muscle tissue since the replacement of muscle tissue by connective tissue (fibrosis) is a major cause of permanent muscle weakness. Accordingly, RyCal compounds can be tested for inhibition of these changes. Conventional histology techniques will allow to assess variability in fiber size, split fibers, and centralized nuclei.

[00184] Fiber typing by histochemistry in skeletal muscles: Historically, the most widely used classification of fiber types is based on the mATPase (myofibrillar adenosine triphosphatase) activity by histochemistry which distinguishes between type I (low activity) and type II (high activity) fibers (Brooke et al., 1970). By characterizing the pH lability of the mATPase, type II fibers were further subdivided into IIA and IIB fibers. Additional fiber type characterization with physiologic, histochemical, and ultrastructural methods has revealed: type I, intermediate slow-twitch oxidative; type IIA, red fast-twitch oxidative-glycolytic; and type IIB, white fast-twitch glycolytic fibers. Quantitative histochemistry can be used to determine mATPase, succinate dehydrogenase, and α-glycerophosphate dehydrogenase and cross-sectional areas in MHC-based fiber type changes in oxidative and glycolytic capacities resulting from sustained exercise and/or RyCal compound treatment. To determine myofiber ATPase activity, a protocol using 10 μm thick frozen sections which are

preincubated for 5 mins under acidic or 12 mins under alkaline conditions can be used. Succinate dehydrogenase staining will allow characterizing activity and distinction between muscle fibers. Changes in oxidative muscle fiber types and improvement after treatment for myotonia have been reported in mice previously (Reininghaus et al., 1988).

[00185] Fiber typing by immunocytochemistry in skeletal muscles: To quantitate changes in myonuclear number and location and to distinguish from nuclei of interstitial cells, morphometry by fluorescence microscopy using stains for nuclei (DAPI) and basement membrane (anti-laminin) will be applied. This technique will be particularly important to precisely determine cross-sectional area and number of nuclei in muscle fibers. Moreover, the technique allows for combined immunocytochemistry with the following affinity-purified antibodies: C-terminal RyR2-5029, phospho-epitope-specific RyR1-p2844 (Wehrens et al., 2004), calstabin1 (FKBP12) (Jayaraman et al., 1992), isoform-specific myosin heavy chain (MHC) antibodies (Rivero et al. 1999), and α-actinin (Ruehr et al., 2003) using a previously established protocol with 10 μm thick cryostat sections of EDL or soleus muscles (Moschella et al., 1995). This approach will allow us to classify myofibers and phenotypic changes according to MHC content, metabolic activity, fiber size, RyR1 PKA phosphorylation, and calstabin1 binding occuring from sustained exercise and/or RyCal compound treatment. Further, the fiber type and immunocytochemistry data will be correlated to isolated skeletal muscle function, general histologic data, histochemistry, RyR1 single-channel function, and mitochondria data. Using immunohistochemical staining, fiber type-specific improvement of calstabin1 binding to RyR1 and RyR1 PKA phosphorylation can be determined in the presence or absence, or after RyCal compound treatment in α-actinin positive compartments (Z-disk), and for increased calpain expression in the myofibrillar area (Z disks; compartment containing RyR1 channels) of muscle fibers.

[00186] Skeletal muscle oxidative capacity: Muscle oxidative activity correlates with skeletal muscle adoption to aerobic exercise. As a general test, fast- and slow-twitch skeletal muscle oxidative enzyme activity will be assessed by spectrophotometric assessment of the citric acid cycle and citrate synthase activity in muscle homogenates. Activity as determined as the complex from coenzyme A and oxaloacetate is expected to be increased as reported by a group using a similar protocol (Evangelista et al., 2003). This test will be used to confirm changes in oxidative capacity seen by histochemistry.

[00187] Improvement of creatine kinase (CK) plasma levels: Creatine kinase (CK) and lactate dehydrogenase (LDH) plasma concentrations (Santos et al., 2004; Thompson et al., 2004), can be determined as indicators or muscle damage and inflammation after exhaustive exercise and to determine effect due to RyCal compound treatment..

[00188] RyR1 composition and function in white blood cells: RYR1 in immune cells functions as a Ca^{2+} release channel during B- or T-cell receptor-stimulated activation (Sei t al., 2002; Kraev et al., 2003). For functional analysis, peripheral white blood cells are isolated from mouse blood samples by centrifugation. Leukocytes (10^6/ml) are loaded with 1 μm acetoxy-methyl ester of fluo-3 (Molecular Probes, Eugene, OR) by incubation for 30 mins at 25°C and caffeine sensitivity of intracellular Ca^{2+} release is tested. Compostion and PKA phosphorylation of the RyR1 channel complex will be characterized. Investigating RyR1 in white blood cells will allow monitoring temporal changes during sustained exercise and RyCal compound treatment *in vivo*.

[00189] **Genetically modified mice**

[00190] The RyR1 macromolecular signaling complex plays a key role in modulating activation of the channel and excitation-contraction coupling by the sympathetic nervous system. In the RyR1 complex mAKAP targets PKA and the phosphodiesterase PDE4D3 to the channel and the phosphatase PP1 is targeted to the channel by the targeting protein spinophilin. This signaling module controls PKA phosphorylation of RyR2 at Ser2843 as part of the "fight-or-flight" stress response. During normal exercise 2-3 of the four Ser2843 PKA phosphorylation sites in each tetrameric RyR1 channel are transiently PKA phosphorylated resulting in increased activity of the RyR1 channel.

[00191] Activation of the RyR1 due to PKA phosphorylation occurs because PKA phosphorylation decreases the binding affinity of the stabilizing protein calstabin1 (FKBP12) for the channel resulting in increased sensitivity of the channel to Ca^{2+}-dependent activation. PDE4D3 in the RyR1 macromolecular signaling complex plays a protective role against PKA hyperphosphorylation and forms a negative feedback loop during PKA activation. The phosphodiesterases in the RyR1 complex by rapidly degrading local cAMP and thereby terminating channel activation by PKA. Mice that are deficient in PDE4D3 or calstabin1 in the RyR1 complex will be tested for accelerated muscle fatigue. Thus, both calstabin1 and PDE4D3 in the RyR2 complex can be thought of as being "protective" against muscle

dysfunction during excessive exercise or stress. Thus additional components of the RyR1 macromolecular complex are protective against fatigue as these molecules could potentially be novel therapeutic targets and/or identify adverse pharmaceutical agents for preventing fatigue during intense stress in warfighters.

[00192] The muscle-specific genetic mouse model HSA^{LR} of myotonic dystrophy type 1 (DM1) has a DM-like phenotype. Importantly, the HSA^{LR} myotonic phenotype includes variable degress of histopathological signs of muscle degeneration and repair, which correspond to the expression of more toxic, long or less, toxic short repeat variants. In addition to wild-type mice, susceptibility to muscle fatigue will be investigated using swimming and treadmill running protocols. In a subgroup of mice, implantable telemetry devices will be implanted two weeks before the mice are subjected to an exercise-stress protocol as described. Upon completion of the experiment, mice will be sacrificed and muscles will be flash-frozen in liquid nitrogen or further processed for histological assays. Specific muscle types are carefully dissected under stereoscope vision and flash frozen in liquid nitrogen or examined by histology and immunohistochemistry. In all tissue samples, RyR1 PKA phosphorylation, levels of the components of the RyR2 macromolecular complex including calstabin1, PKA, RII, mAKAP, PP1, spinophilin, PDE4D3, CaMKII, and single channel properties will be examined. The following properties are determined: Ca^{2+} sensitivity of activation and inhibition, Mg^{2+} inhibition, and changes from *in vivo* RyCal treatment. At conclusion of each single channel experiment ryanodine will be applied to the channel to confirm RyR identity. The HSA^{LR} mouse will allow to test beneficial effects of RyCal compounds in an extreme model of genetic muscle fatigue and dystrophy.

[00193] Susceptibility to muscle fatigue can be investigated using swimming and treadmill running protocols. Implantable telemetry devices can be implanted two weeks before the mice are subjected to an exercise-stress protocol. Upon completion of the experiment, mice are sacrificed and muscles are flash-frozen in liquid nitrogen. Upon completion of each experiment, muscles can be dissected and will also be examined by histology and Western blotting. In all tissue samples, RyR1 PKA phosphorylation, levels of the components of the RyR2 macromolecular complex including calstabin1, PKA, RII, mAKAP, PP1, spinophilin, PDE4D3, CaMKII, and single channel properties can be examined. The following properties can be determined: Ca^{2+} sensitivity of activation and

inhibition, Mg^{2+} inhibition, response to PKA phosphorylation. At conclusion of each single channel experiment ryanodine is applied to the channel to confirm RyR identity.

[00194] RyCal compounds prevent muscle fatigue and muscle degeneration

[00195] In certain aspects, the invention provides that RyCal compounds can restore normal function to hyperphosphorylated RyR1. Furthermore, use of PDE4D knockout and FKBP12 haploinsufficient mice allows determination whether RyCal compound prevent muscle fatigue.

[00196] The foregoing discussion established two animal models (mouse and rat) of muscle dysfunction and fatiguing, which result from sustained forms of exercise. The models can provide important clues if chronic activation of the sympathetic nervous system resulting from sustained exercise and stress cause a critical defect in the RyR1 Ca^{2+} release channel. Previous experiments in a heart failure model that results in chronic sympathetic hyperactivity have established a critical defect in RyR1 contributing to accelerated muscle fatigue. RyCal compounds have beneficial effects to inhibit muscle fatigue during sustained exercise capacity, and isolated slow-and fast-twitch skeletal muscle function in the investigated fatigue models. Catecholamine-induced muscle fatigue has been established in rat and mouse hearts failure models earlier and therefore we will be able to apply similar techniques to test for muscle fatigue following sustained animal exercise protocols.

[00197] Maintenance of muscle performance during sustained activation of the sympathetic nervous system, for example but not limited to combat, requires a maximal rate of intracellular SR Ca^{2+} cycling. Chronic maximal stress results in permanent activation of the sympathetic nervous system potentially causing RyR1 hyperphosphorylation and intracellular Ca^{2+} leak. In skeletal muscles, intracellular Ca^{2+} leak gradually causes a myopathy characterized by significantly reduced duration and maximal power of peak performance as well as accelerated fatigue by additional ATP consumption of SR Ca^{2+} ATPase pumps that compensate for uncontrolled SR Ca^{2+} leak. SR Ca^{2+} leak is unique since it is a direct cause of muscle fatigue intrinsic to myofibers which is not reversible in the acute setting. A drug like any RyCal compound which fixes the SR Ca^{2+} leak by binding calstabin1 to the channel and stabilizing the closed state even during stress therefore helps to prevent accelerated fatigue development and promotes longer performance despite sustained stress (Wehrens, 2005). The pharmacotherapy is unique since it targets a central fatigue

mechanism and potentially prevents toxic effects of intracellular Ca^{2+} leak. Moreover the molecular mechanism of this pharmocotherapy is unique, since it treats a specific defect contributing to muscle fatigue and since the mechanism of RyCal action is stabilization of normal RyR1 channel closure by increasing the calstabin1 binding affinity, which is distinct from historical approaches that block ion channel function.

[00198] More recently, SR Ca^{2+} leak was documented in myofibers following intense exercise and in a model of muscular dystrophy,(Wang et al., 2005), possibly due to defective skeletal ryanodine receptors (RyR1s). Also, chronic activation of the sympathetic nervous system (SNS) in the context of heart failure promotes intrinsic skeletal muscle (SM) fatigue due to depletion of the phosphodiesterease PDE4D3 from the RyR1 complex, RyR1 PKA hyperphosphorylation at Serine 2844, calstabin1 depletion from the RyR1 complex, and a gain-of-function channel defect (Reiken et al., 2003). RyR1 dysfunction in the skeletal muscle leads to altered local subcellular Ca^{2+} release events and impaired global calcium transients (Ward et al., 2003). In the context of chronic exercise, there is evidence indicating that changes in the RyR1 macromolecular complex, namely depletion of PDE4D3 from the RyR1 complex, RyR1 PKA hyperphosphorylation at Serine 2844, and calstabin1 depletion from the RyR1 complex are related in a time-dependent and activity-dependent manner with repeated intense exercise in a mouse model. These biochemical changes in the RyR1 macromolecular complex regulation and function are stable following prolonged exercise and recover slowly over days to weeks. Thus RyR1 Ca^{2+} leak limits peak muscle performance and mediates muscle damage during prolonged, stressful exercise.

[00199] **Molecular mechanisms of muscle fatigue**

[00200] The hypotheses that muscle fatigue is due to lactic acid accumulation in the cytoplasm and potassium ion accumulation in T-tubules have largely been set aside and attention has shifted to the study of metabolic and mitochondrial regulation and signaling pathways during chronic exercise (Lin, Wu et al. 2002; Wu, Kanatous et al. 2002; Wang, Zhang et al. 2004). These alternative explanations, while important, are unlikely to directly address the underlying abnormalities in ECC observed in fatigued muscle (Berchtold, Brinkmeier et al. 2000). Described herein is the regulation of the skeletal calcium release channel, RyR1, during chronic or high intensity exercise. The remodeling of the RyR1 macromolecular complex during chronic exercise, consisting of PKA

hyperphosphorylation at Ser2844, PDE4D3 depletion, and calstabin1 depletion, likely plays a role in determining muscle fatigue during chronic exercise.

[00201] Exercise promotes numerous positive effects on an organism, from improvement in cardiovascular performance to increased glucose uptake and normalization of fuel metabolism (Goodyear and Kahn 1998; Pollock, Franklin et al. 2000). In heart failure, light exercise training has been shown to improve skeletal muscle strength and reduce fatigue, perhaps through adaptation to more aerobic muscle properties (Minotti, Johnson et al. 1990; Lunde, Sjaastad et al. 2001; Meyer 2006). On the other hand, high intensity exercise, such as that performed by a marathon runner or a long distance cyclist results in significant muscle damage and can impair task performance for days or weeks after a single event (O'Reilly, Warhol et al. 1987; Balnave and Thompson 1993; Komulainen and Vihko 1994).

[00202] Described herein is also a mouse model of intense physiological exercise to examine the changes in RyR1 function and ECC experienced by elite athletes, soldiers, or others under intense stressful activity. By combining daily swimming with level treadmill running assays, a physiological exercise regimen was constructed that did not exclusively involve isometric or eccentric contraction of the hind limb. While this resulted in less dramatic evidence of exercise-induced muscle damage than pure eccentric contractions, the data presented are more readily generalized.

[00203] Biochemical changes were identified in the RyR1 macromolecular complex consistent with leaky calcium release channels. Single channel bilayer data confirmed a leaky phenotype of the RyR1 channels from chronically exercised hind limb muscle, with elevated open probabilities in the chronically exercised group at resting calcium levels compared to sedentary controls. In two mouse genetic models replicating aspects of the biochemical changes in the RyR1 complex, namely muscle-specific deficiency of calstabin1 (cal1-/-) and deficiency of PDE4D3 (PDE4D-/-), exercise defects were identified. The role of calstabin1 depletion was assessed in another way by pharmacologically rebinding the stabilizing subunit to RyR1 with the Ca^{2+} channel stabilizer S107. Calstabin1 rebinding to RyR1, induced by S 107 resulted in improved exercise capacity, as measured by treadmill failure times, over the same 21 day time course that depleted calstabin1 in the vehicle treated mice. The lack of an effect of S107 on *cal1-/-*

mice provides evidence that the molecular mechanism of S107 is indeed through calstabin1 rebinding to RyR1.

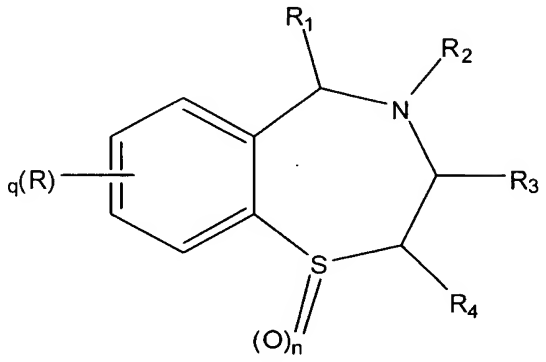
[00204] *In vitro* fatigue protocols on intact isolated muscles suffer from the limitation that force declines are largely limited by hypoxia (Zhang, Bruton et al. 2006). Therefore, single FDB muscle fibers were isolated from sedentary and chronically exercised mice with and without S107 for assessment of the decline in tetanic Ca^{2+} during fatigue. Exercised fibers with calstabin rebound were relatively protected against fatigue (Fig. 13). The effect of S107 did not appear to be due to a shift in the fiber kinetics to slower calcium cycling.

[00205] S107 corrected the leak in RyR1 from chronically exercised mice as measured in a lipid bilayer at low resting Ca^{2+} levels. Ca^{2+} leak resulting from the overactive skeletal ryanodine receptors was not directly visualized in isolated muscle fibers, as calcium sparks were infrequent under all conditions tested, which is consistent with most reports that sparks in skeletal muscle are rare except under certain highly pathological conditions such as hypoosmotic shock or muscular dystrophy (Isaeva, Shkryl et al. 2005; Rios 2005; Wang, Weisleder et al. 2005).

[00206] Numerous hypotheses present themselves for how alterations in RyR1 Ca^{2+} leak could result in muscle damage. These data do not identify one muscle damage pathway solely responsible for the physiological effects seen, however, they implicate a role for calpain activation during chronic, and/or high intensity exercise. Several groups have demonstrated that calpain activation is a major mechanism for exercise-induced muscle damage(Belcastro 1993; Spencer and Mellgren 2002). As described herein, calpain activation in isolated EDL muscle was elevated following chronic exercise, but reduced by treatment with S107, suggesting that correction of the leaky RyR1 may protect against calpain activation (Fig. 15). With a potential contribution of other Ca^{2+}- dependent pathways such as caspases, calmodulin, or calmodulin-dependent kinases to the damage induced by leaky ryanodine receptors, the present data suggest a mechanism by which local elevation of cytosolic Ca^{2+} could lead to damage. The hypothesis that ryanodine receptor-induced leak can cause muscle damage was further supported by evidence of reduced muscle damage, as measured by serum creatine kinase, in the S107 treated mice (Fig. 15). The data described herein shows that changes in the RyR1 macromolecular complex producing a leaky phenotype during chronic, high intensity exercise impairs exercise performance.

Compounds

[00207] In one aspect, the present invention provides methods and uses which comprise administering compounds of Formula I:



wherein,

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be optionally substituted;

R_1 is selected from the group consisting of H, oxo, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_2 is selected from the group consisting of H, $-C(=O)R_5$, $-C(=S)R_6$, $-SO_2R_7$, $-P(=O)R_8R_9$, $-(CH_2)_m-R_{10}$, alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be optionally substituted;

R_3 is selected from the group consisting of H , $-CO_2Y$, -C(=O)NHY, acyl, -O-acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally

substituted; and wherein Y is selected from the group consisting of H, alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl, and wherein each alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_4 is selected from the group consisting of H, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_5 is selected from the group consisting of $-NR_{15}R_{16}$, $-(CH_2)_zNR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-C(=O)NHNR_{15}R_{16}$, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-CH_2X$, acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted, and wherein z is 1, 2, 3, 4, 5, or 6;

R_6 is selected from the group consisting of $-OR_{15}$, $-NHNR_{15}R_{16}$, -NHOH, $-NR_{15}R_{16}$, $-CH_2X$, acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_7 is selected from the group consisting of $-OR_{15}$, $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-CH_2X$, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_8 and R_9 independently are selected from the group consisting of OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_{10} is selected from the group consisting of $-NR_{15}R_{16}$, OH, $-SO_2R_{11}$, $-NHSO_2R_{11}$, $C(=O)(R_{12})$, $NHC=O(R_{12})$, $-OC=O(R_{12})$, and $-P(=O)R_{13}R_{14}$;

R_{11}, R_{12}, R_{13}, and R_{14} independently are selected from the group consisting of H, OH, NH_2, $-NHNH_2$, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl,

cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

X is selected from the group consisting of halogen, -CN, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-NR_{15}R_{16}$, $-OR_{15}$, $-SO_2R_7$, and $-P(=O)R_8R_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH_2, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted;

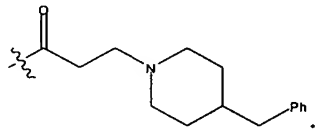
the nitrogen in the benzothiazepine ring may optionally be a quaternary nitrogen; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof.

[00208] In certain embodiments of Formula I, it is provided that when q is 0 and n is 0, then R_2 is not H, Et, $-C(=O)NH_2$, (=O)NHPh, -C(=S)NH-nButyl, $-C(=O)NHC(=O)CH_2Cl$, $-C(=O)H$, -C(=O)Me, -C(=O)Et, $-C(=O)CH=CH_2$, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 0 and n is 1 or 2, then R_2 is not -C(=O)Me, -C(=O)Et, $-S(=O)_2Me$, or $-S(=O)_2Et$;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)Ph, $-S(=O)_2Me$, or $-S(=O)_2Et$; and

further provided that when q is 1, n is 0, and R is OCT_3, OH, C_1-C_3 alkoxyl at the 7 position of the benzothiazepene ring, then R_2 is not H, $-C(=O)CH=CH_2$, or 

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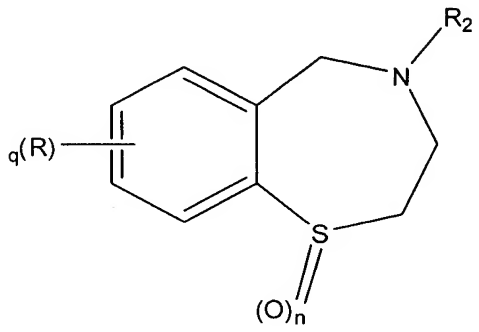
[00209] In one embodiment, the present invention provides methods and uses which comprise administering compounds of Formula I, as described above, with the proviso that said compound is not S24 or S68.

[00210] In one embodiment, the present invention provides methods and uses which comprise administering compounds of Formula **I-a:**



(I-a)

wherein:

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted or unsubstituted;

R_2 is selected from the group consisting of H, $-C=O(R_5)$, $-C=S(R_6)$, $-SO_2R_7$, $-P(=O)R_8R_9$, $-(CH_2)_m-R_{10}$, alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be substituted or unsubstituted;

R_5 is selected from the group consisting of $-NR_{15}R_{16}$, $-(CH_2)_zNR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-C(=O)NHNR_{15}R_{16}$, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-CH_2X$, acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted, and wherein z is 1, 2, 3, 4, 5, or 6;

R_6 is selected from the group consisting of $-OR_{15}$, $-NHNR_{15}R_{16}$, -NHOH, $-NR_{15}R_{16}$, $-CH_2X$, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl;

wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_7 is selected from the group consisting of H, $-OR_{15}$, $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-CH_2X$, alkyl, akenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, akenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_8 and R_9 independently are selected from the group consisting of -OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_{10} is selected from the group consisting of $-NR_{15}R_{16}$, OH, $-SO_2R_{11}$, $-NHSO_2R_{11}$, $-C(=O)R_{12}$, $-NH(C=O)R_{12}$, $-O(C=O)R_{12}$, and $-P(=O)R_{13}R_{14}$;

m is 0, 1, 2, 3, or 4;

R_{11}, R_{12}, R_{13}, and R_{14} independently are selected from the group consisting of H, OH, NH_2, $-NHNH_2$, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

X is selected from the group consisting of halogen, -CN, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-NR_{15}R_{16}$, $-OR_{15}$, $-SO_2R_7$, and $-P(=O)R_8R_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH_2, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted or unsubstituted;

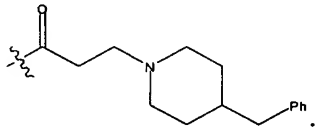
the nitrogen in the benzothiazepine ring may be optionally a quaternary nitrogen; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof.

[00211] In one embodiment, it is provided that when q is 0 and n is 0, then R_2 is not H, Et, -C(=O)NH_2, (=O)NHPh, -C(=S)NH-nButyl, -C(=O)NHC(=O)CH_2Cl, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)CH=CH_2, -S(=O)$_2$Me, or -S(=O)$_2$Et;

further provided that when q is 0 and n is 1 or 2, then R_2 is not -C(=O)Me, -C(=O)Et, -S(=O)$_2$Me, or -S(=O)$_2$Et;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)Ph, -S(=O)$_2$Me, or -S(=O)$_2$Et; and

further provided that when q is 1, n is 0, and R is OCT_3, OH, C_1-C_3 alkoxyl at the 7 position of the benzothiazepene ring, then R_2 is not H, -C(=O)CH=CH_2, or 

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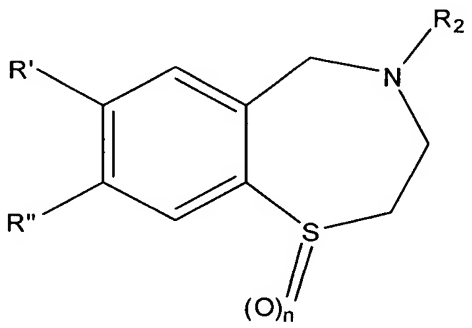
[00212] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-a**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH_2, -NO_2, -CN, -CF_3, -OCF_3, -N_3, -S(=O)$_2$$C_1$-$C_4$alkyl, -S(=O)$C_1$-$C_4$alkyl, -S-$C_1$-$C_4$alkyl, -OS(=O)$_2$$CF_3$, Ph, -$NHCH_2$Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00213] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-a**, wherein R_2 is selected from the group consisting of –C=O(R_5), –C=S(R_6), -SO_2R_7, –P(=O)R_8R_9, and -$(CH_2)_m$-R_{10}.

[00214] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula **I-b**:



(I-b)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, -NH_2, -NO_2, -CN, -CF_3, -OCF_3, -N_3, -SO_3H, -S(=O)$_2$alkyl, -S(=O)alkyl, -OS(=O)$_2$$CF_3$, acyl,

alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

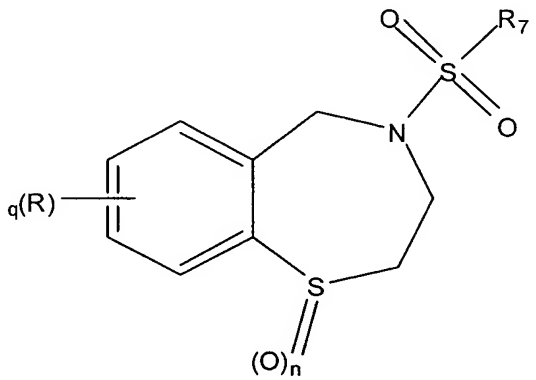
R_2 and n are as defined in compounds of formula **I-a** above;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00215] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-b**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00216] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-b**, wherein R_2 is selected from the group consisting of $-C=O(R_5)$, $-C=S(R_6)$, $-SO_2R_7$, $-P(=O)R_8R_9$, and $-(CH_2)_m-R_{10}$.

[00217] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds formula of **I-c**:



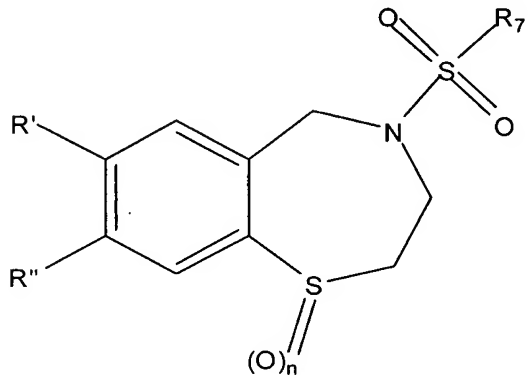
(I-c)

wherein each R, R_7, q, and n is as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00218] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-c**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00219] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-c**, wherein R_7 is selected from the group consisting of -OH, $-NR_{15}R_{16}$, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, akenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00220] In a further embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-d:**



(I-d)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

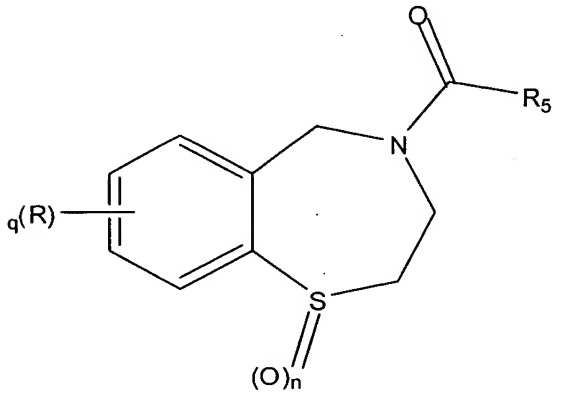
R_7 and n are as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00221] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-d**, wherein R' and R" are

independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00222] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-d**, wherein R_7 is selected from the group consisting of -OH, $-NR_{15}R_{16}$, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, akenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00223] In one embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-e**:



(I-e)

wherein each R, R_5, q and n is as defined compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

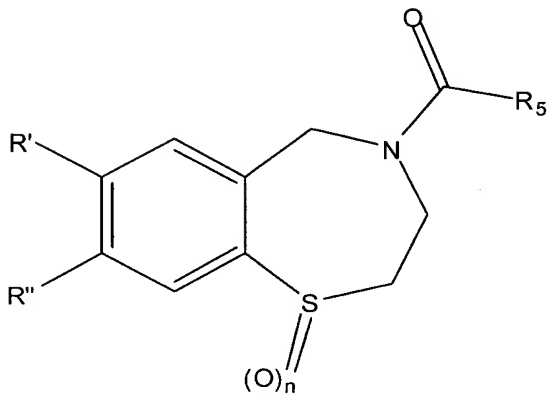
[00224] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-e**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00225] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-e**, wherein R_5 is selected from the group consisting of $-NR_{15}R_{16}$, $-(CH_2)_zNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkyl, alkenyl, aryl,

cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00226] In some embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-e**, wherein R_5 is an alkyl substituted by at least one labeling group, such as a fluorescent, a bioluminescent, a chemiluminescent, a colorimetric and a radioactive labeling group. A fluorescent labeling group can be selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[00227] In another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-f**:



(I-f)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

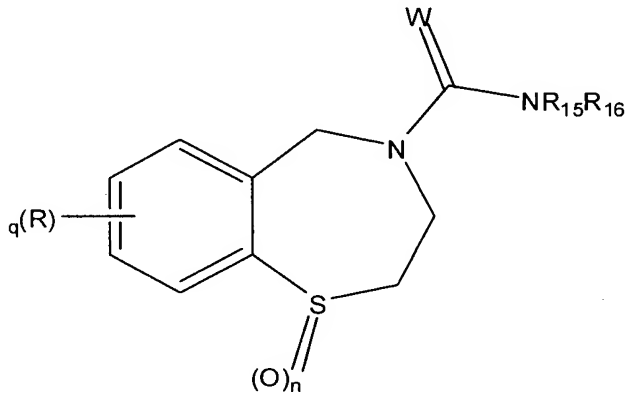
R_5 and n are as defined in compounds of formula **I-a** above;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00228] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-f**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00229] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-f**, wherein $-(CH_2)_zNR_{15}R_{16}$, selected from the group consisting of $-NR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00230] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-g**:



(I-g)

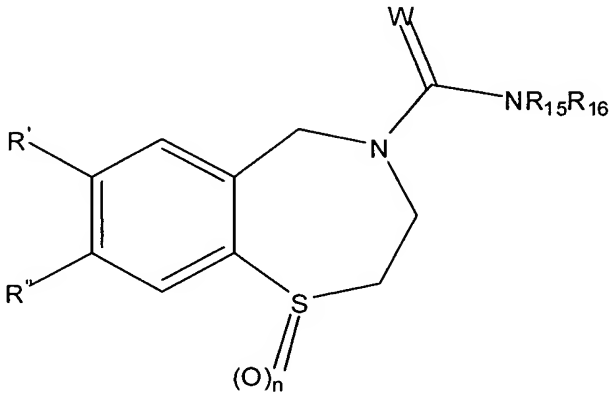
wherein W is S or O; each R, R_{15}, R_{16}, q, and n is as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00231] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00232] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein R_{15} and R_{16} independently are selected from the group consisting of H, OH, NH_2, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted.

[00233] In some embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein W is O or S.

[00234] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-h**:



(I-h)

wherein W is S or O;

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{15}, R_{16} and n are as defined in compounds of formula **I-a** above;

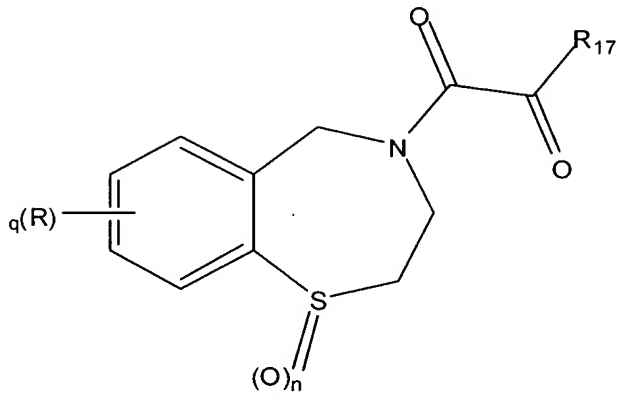
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00235] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-h**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00236] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-h**, wherein R_{15} and R_{16} independently are selected from the group consisting of H, OH, NH_2, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted.

[00237] In some embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein W is O or S.

[00238] In a further embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-i**:



(I-i)

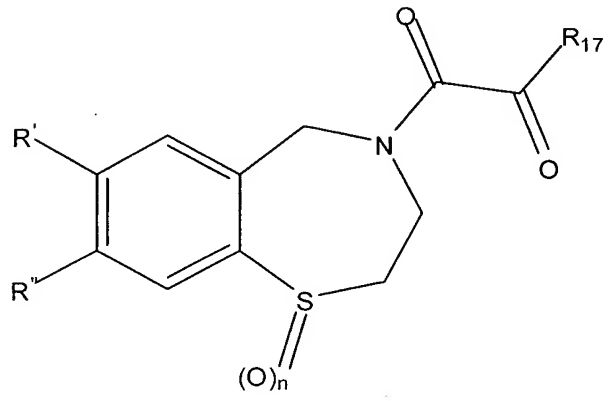
wherein R_{17} is selected from the group consisting of $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

each R, q, and n is as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00239] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-i**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00240] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-i**, wherein R_{17} is $-NR_{15}R_{16}$, and $-OR_{15}$. In certain other embodiments, R_{17} is -OH, -OMe, -NEt, -NHEt, -NHPh, $-NH_2$, or -$NHCH_2$pyridyl.

[00241] In one embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-j**:



(I-j)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{17} is selected from the group consisting of $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each

alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

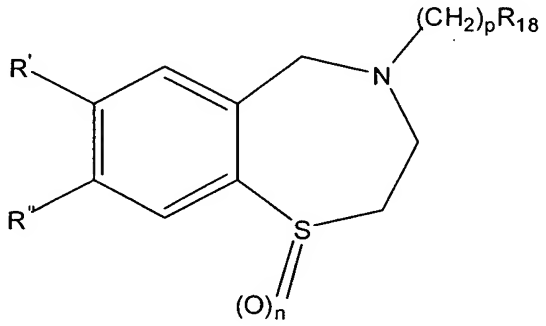
n is as defined in compounds of formula **I-a**; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00242] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-j**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00243] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-j**, wherein R_{17} is $-NR_{15}R_{16}$ or $-OR_{15}$. In certain other embodiments, R_{17} is -OH, -OMe, -NEt, -NHEt, -NHPh, $-NH_2$, or -$NHCH_2$pyridyl.

[00244] In another embodiment, the present invention provides methods and uses which comprise administering compounds of formula **I-k**:



(I-k)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{18} is selected from the group consisting of $-NR_{15}R_{16}$, $-C(=O)NR_{15}R_{16}$, $-(C=O)OR_{15}$, $-OR_{15}$, alkyl, aryl, cycloalkyl, heterocyclyl, and at one labeling group; wherein each alkyl, aryl, cycloalkyl, and heterocyclyl may be substituted or unsubstituted;

wherein p is 1, 2, 3, 4, 5, 6, 7, 8 9, or 10;

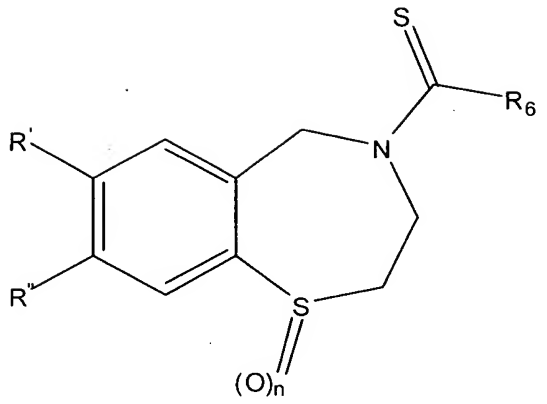
and n is 0, 1, or 2;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00245] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-k**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00246] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-k**, wherein R_{18} is selected from the group consisting of $-NR_{15}R_{16}$, $-(C=O)OR_{15}$, $-OR_{15}$, alkyl, aryl, and at one labeling group; and wherein each alkyl and aryl may be substituted or unsubstituted. In some cases, m is 1, and R_{18} is Ph, C(=O)OMe, C(=O)OH, aminoalkyl, NH_2, NHOH, or NHCbz. In other cases, m is 0, and R_{18} is C_1-C_4 alkyl, such as Me, Et, propyl, and butyl. In yet other cases, m is 2, and R_{18} is pyrrolidine, piperidine, piperazine, or morpholine. In some embodiments, m is 3, 4, 5, 5, 7, or 8, and R_{18} is a fluorescent labeling group selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[00247] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-l**:



(I-l)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

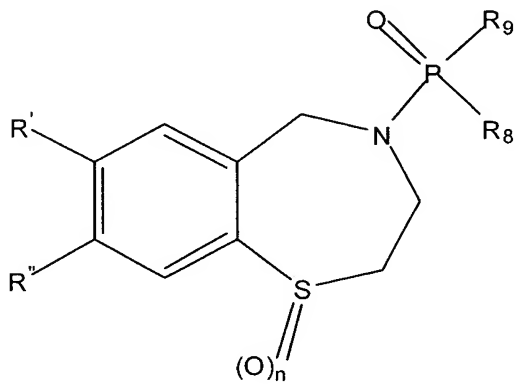
R_6 and n are as defined in compounds of formula **I-a**;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00248] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-l**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00249] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-l**, wherein R_6 is selected from the group consisting of $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, $-OR_{15}$, -NHOH, $-CH_2X$, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted. In some cases, R_6 is $-NR_{15}R_{16}$ such as –NHPh, pyrrolidine, piperidine, piperazine, morpholine, and the like. In some other cases, R_6 is alkoxyl, such as –O-tBu.

[00250] In a further embodiment, the present invention provides methods and uses which comprise administering compounds of formula **I-m:**



(I-m)

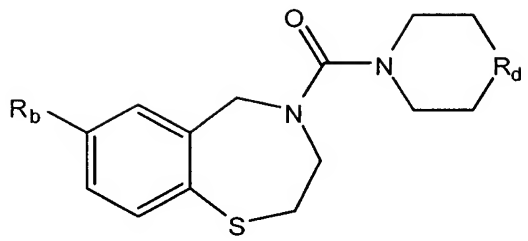
wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_8, R_9 and n are as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00251] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-m**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00252] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-m**, wherein R_8 and R_9 are independently alkyl, aryl, -OH, alkoxyl, or alkylamino. In some cases, R_8 is C_1-C_4 alkyl such as Me, Et, propyl and butyl; and R_9 is aryl such as phenyl.

[00253] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-n**,



I-n

wherein:

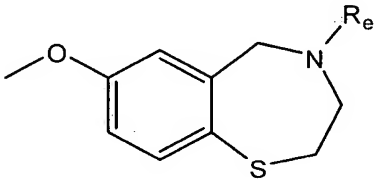
R_d is CH_2, or NR_a; and

R_a is H, –(C_1-C_6 alkyl)-aryl, wherein the aryl is a disubstituted phenyl or a benzo[1,3]dioxo-5-yl group, or an amine protecting group (e.g., a Boc group); and

R_b is hydrogen of alkoxy (e.g., methoxy).

[00254] Representative compounds of Formula **I-n** include without limitation S101, S102, S103, S114.

[00255] In certain other embodiments, the invention provides compounds of Formula **I-o**:



I-o

wherein:

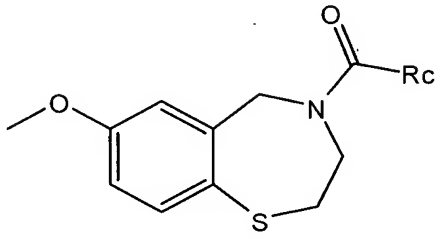
R_e is -(C_1-C_6 alkyl)-phenyl, -(C_1-C_6 alkyl)-C(O)R_b, or substituted or unsubstituted -C_1-C_6 alkyl; and

R_b is –OH or –O-(C_1-C_6 alkyl), and

wherein the phenyl or substituted alkyl is substituted with one or more of halogen, hydroxyl, -C_1-C_6 alkyl, -O-(C_1-C_6 alkyl), -NH_2, -NH(C_1-C_6 alkyl), -N(C_1-C_6 alkyl)$_2$, cyano, or dioxolane.

[00256] Representative compounds of Formula **I-o** include without limitation S107, S110, S111, S120, and S121.

[00257] In certain other embodiments, the invention provides compounds of Formula **I-p**:



I-p

wherein:

R_c is $-(C_1\text{-}C_6$ alkyl)-NH_2, -(C_1-C_6 alkyl)-OR_f, wherein R_f is H or $-C(O)$-(C_1-C_6)alkyl, or $-(C_1$-C_6 alkyl)-NHR_g wherein Rg is carboxybenzyl. Representative compound of Formula **I-p** include without limitation S109, S122, S123.

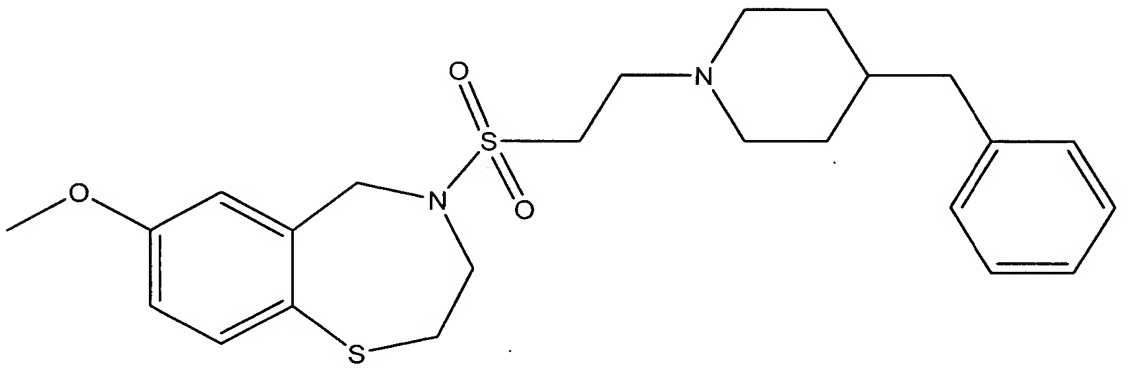
[00258] In non-limiting examples, Formulae-Ia, Ib, Ie, If, Ig, Ih, In are represented by compounds S101, S102, S103. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ii, Ij are represented by compound S104. In a non-limiting example, Formulae Ia, Ib, Io are represented by S107. In a non-limiting example, Formulae Ia, Ib, Ie, If are represented by S108. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ip are represented by S109. In a non-limiting example, Formulae Ia, Ib, Ik, Io are represented by S110. In a non-limiting example, Formulae Ia, Ib, Ik, Io are represented by S111. In a non-limiting example, Formulae Ia, Ib, Ic, Id are represented by S112. In a non-limiting example, Formulae Ia, Ib are represented by S113. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ig, Ih are represented by S114. In a non-limiting example, Formulae Ia, Ib, Ig, Ih, Il are represented by S115. In a non-limiting example, Formulae Ia, Ib, Ig, Ih, are represented by S116. In a non-limiting example, Formulae Ia, Ib, Ie, If are represented by S117. In a non-limiting example, Formulae Ia, Ib, Ie, If are represented by S118. In a non-limiting example, Ia, Ib are represented by S119. In a non-limiting example, Formulae Ia, Ib, Ik, Io are represented by S120. In a non-limiting example, Formulae Ia, Ib, Ik, Io, Ip are represented by S121. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ip are represented by S122. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ip are represented by S123.

[00259] The compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, and I-p, and Formula II can be used in methods that treat or prevent disorders and diseases associated with the RyR receptors.

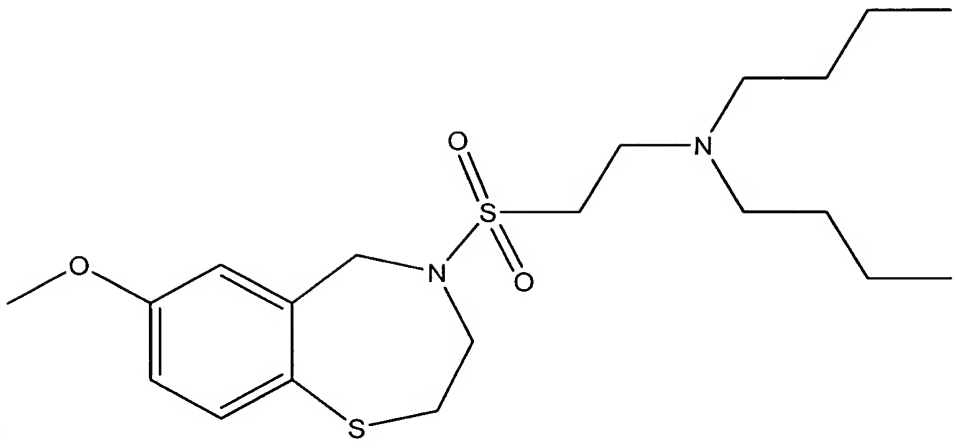
[00260] Examples of such compounds include, without limitation, S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, as herein defined. In certain embodiments, the compounds are isolated and substantially pure.

[00261] In a certain embodiment of the methods the compound is not S4. In another embodiment, the compound is not S7. In another embodiment, the compound is not S8. In another embodiment, the compound is not S10. In another embodiment, the compound is not S20. In another embodiment, the compound is not S24. In another embodiment, the compound is not S25. In another embodiment, the compound is not S26. In another embodiment, the compound is not S27. In another embodiment, the compound is not S36. In another embodiment, the compound is not JTV-519.

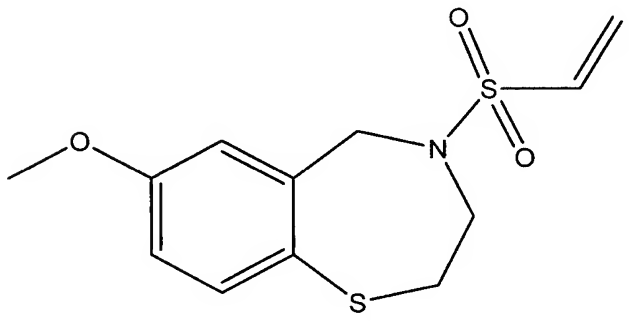
[00262] Certain RyCal compounds of the invention have the following structures:



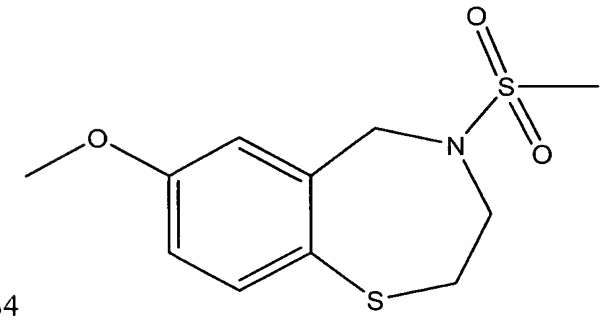
S1



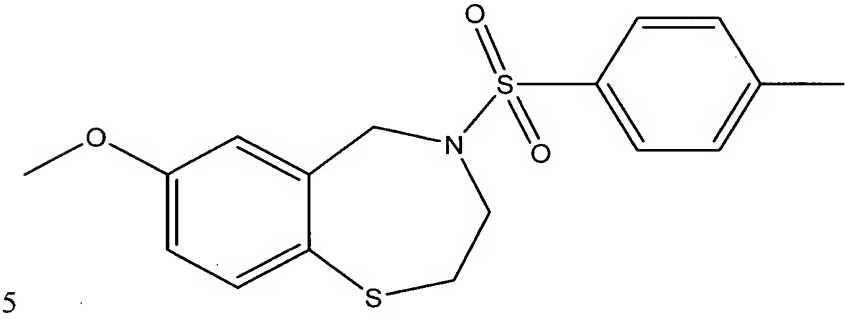
S2



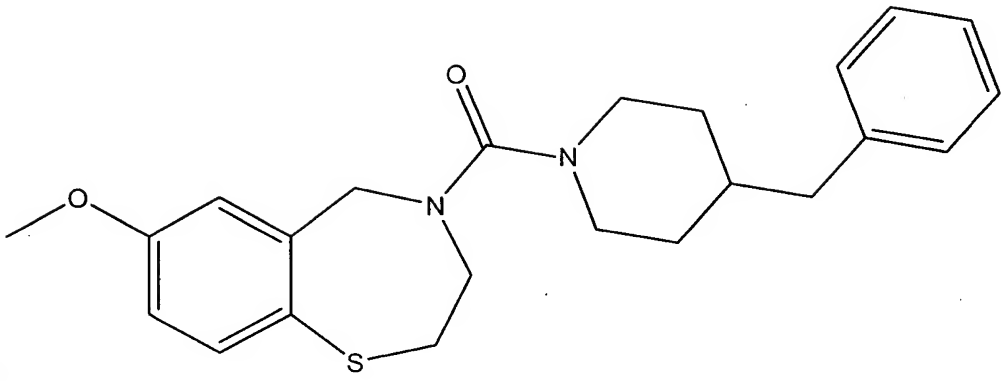
S3



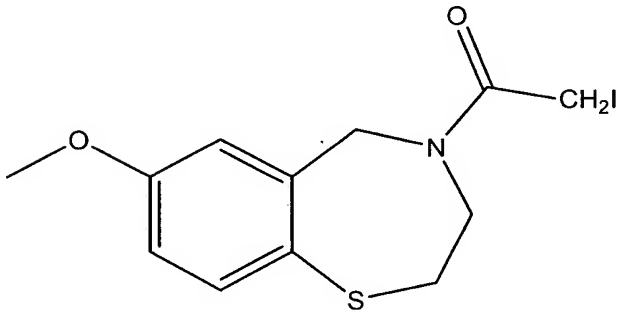
S4



S5



S6



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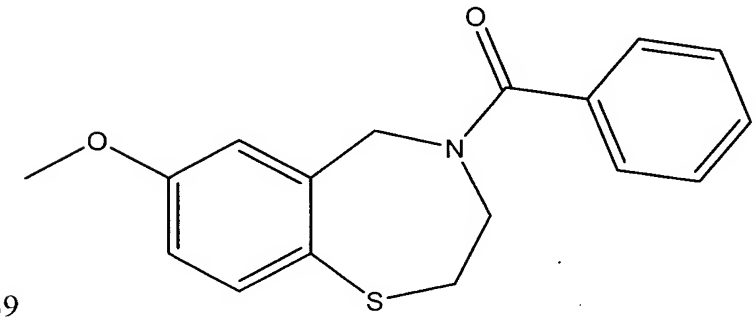

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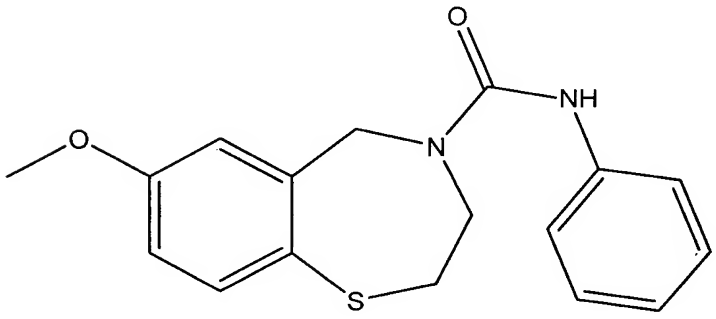
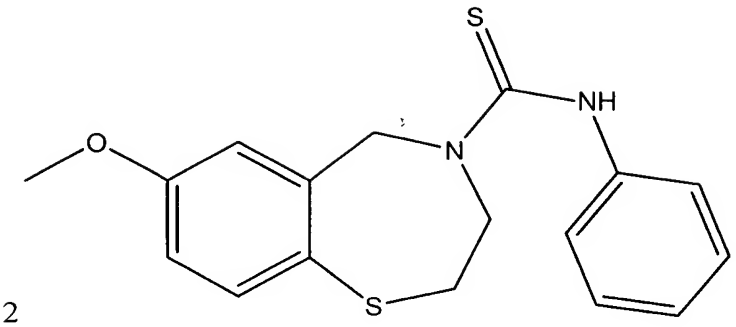


S11

S12



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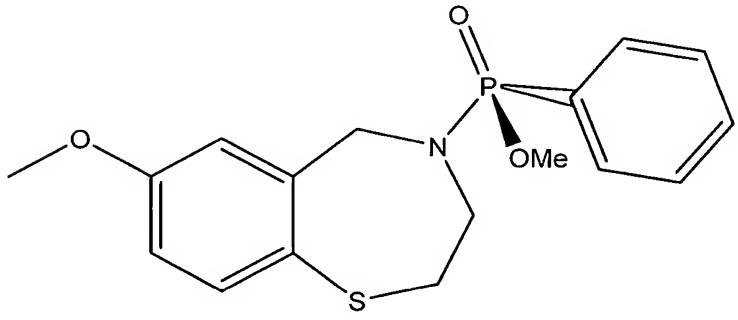

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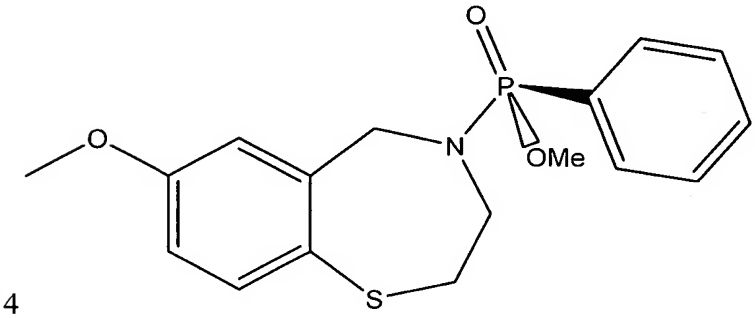

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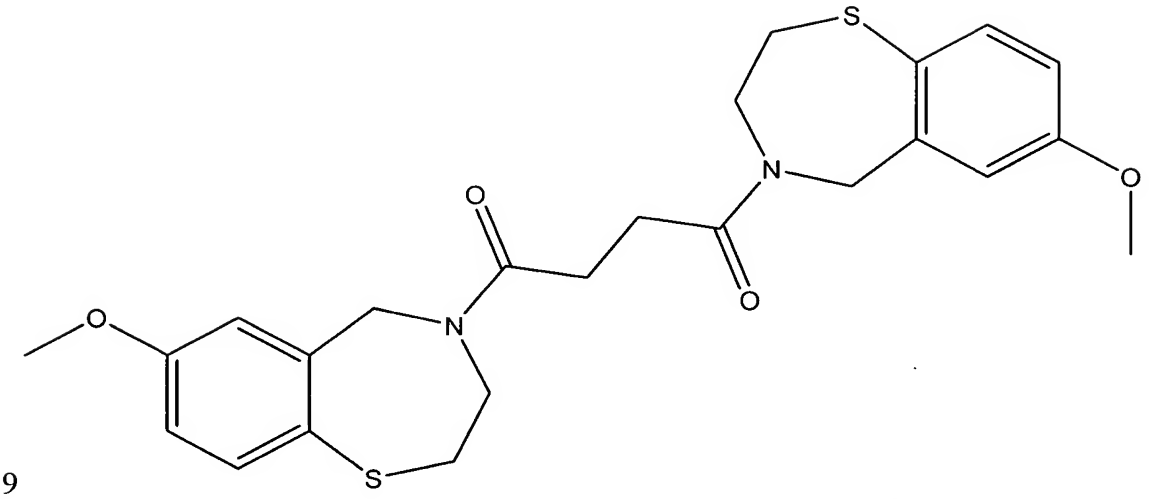

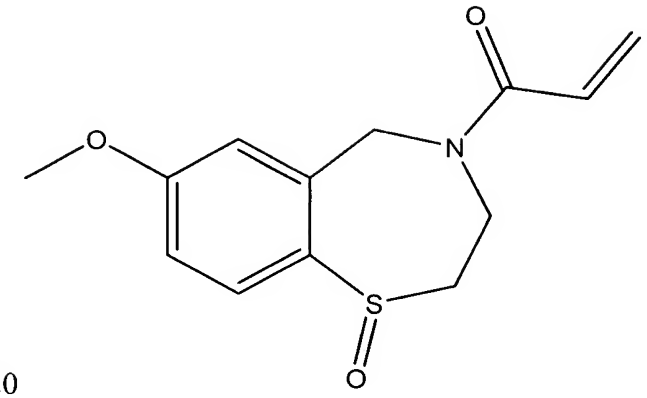

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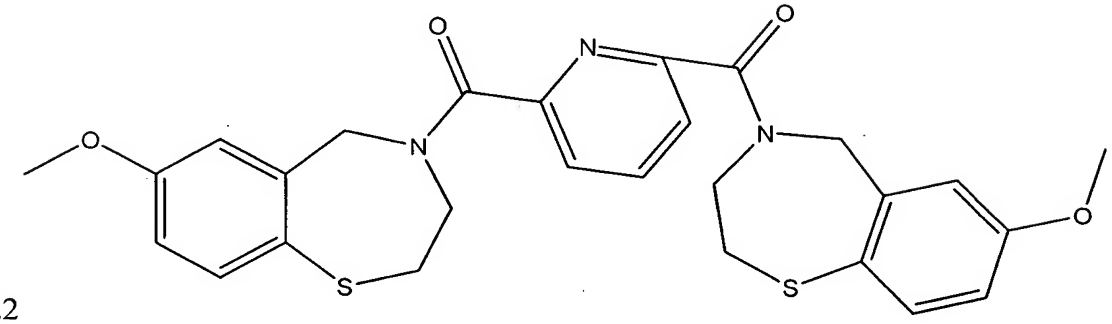

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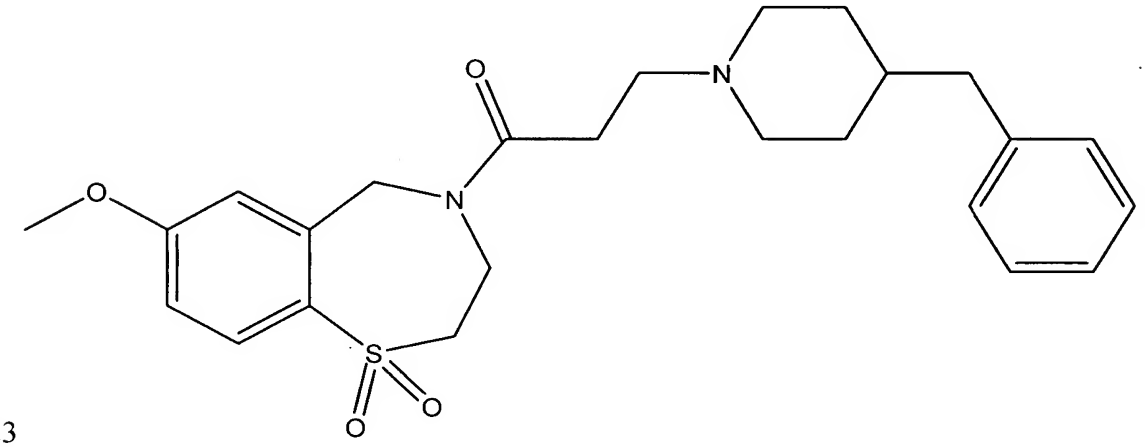
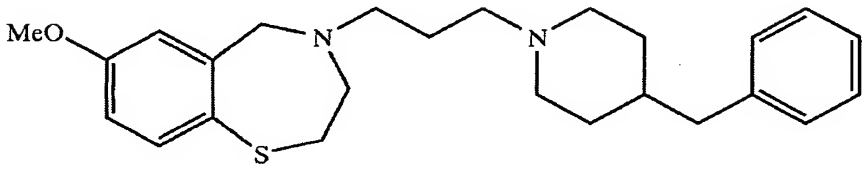


S23

S-24



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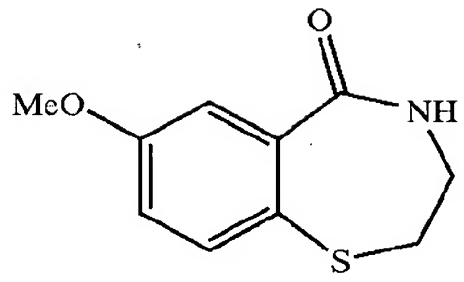

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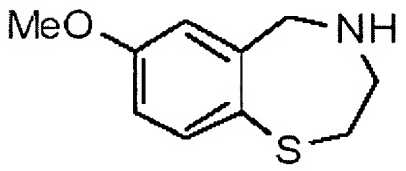

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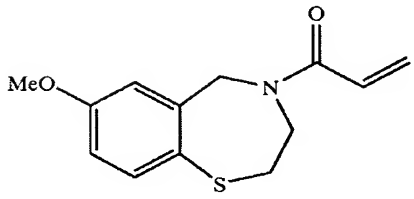

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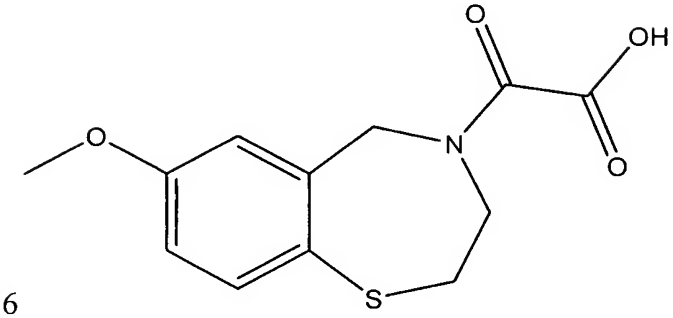

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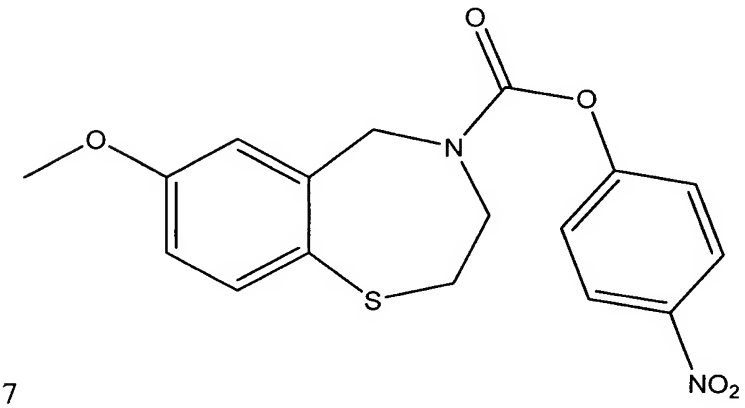

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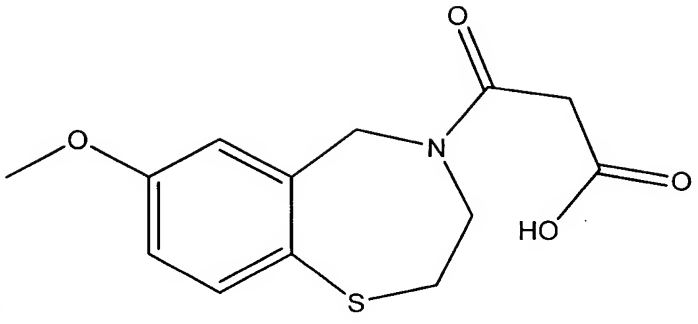

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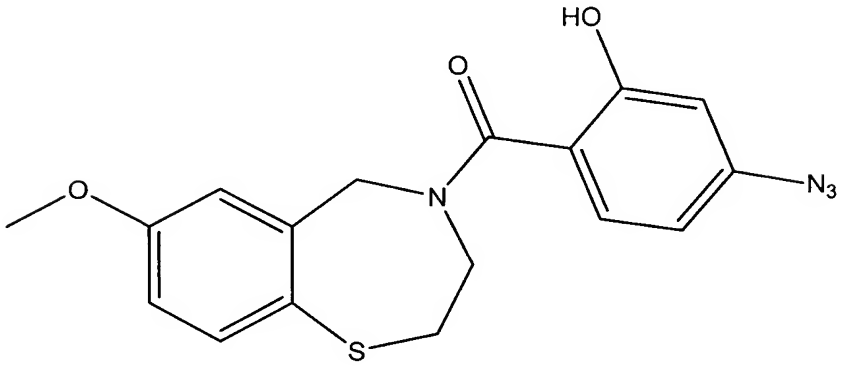

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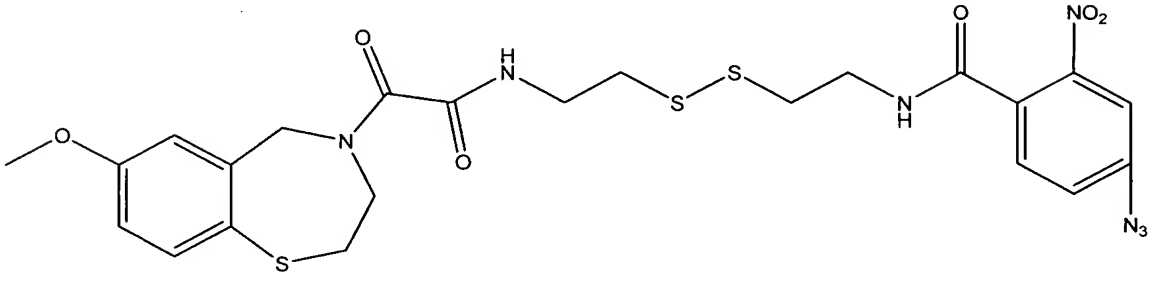

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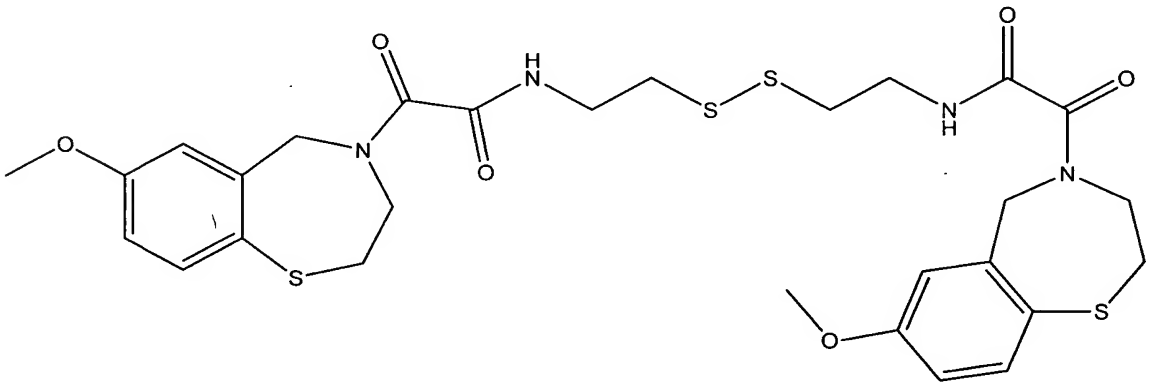

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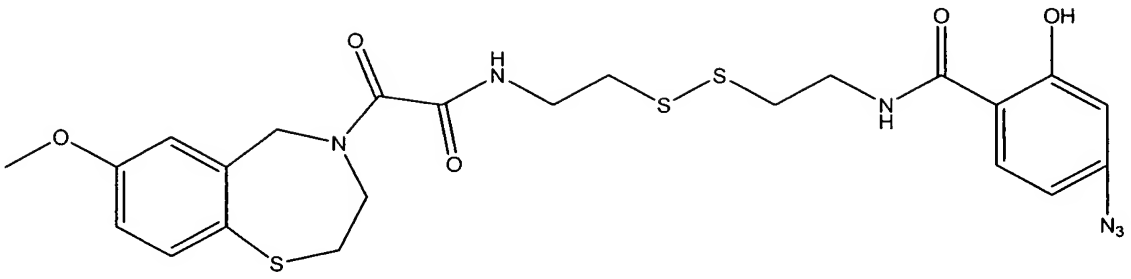

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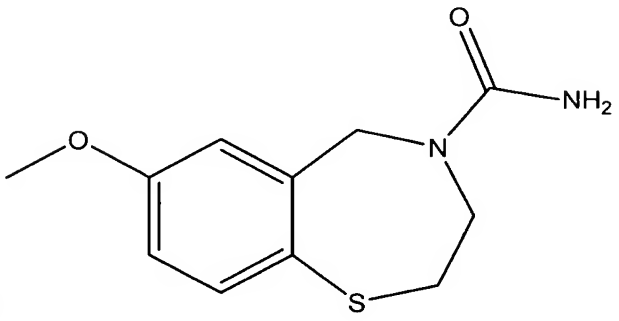

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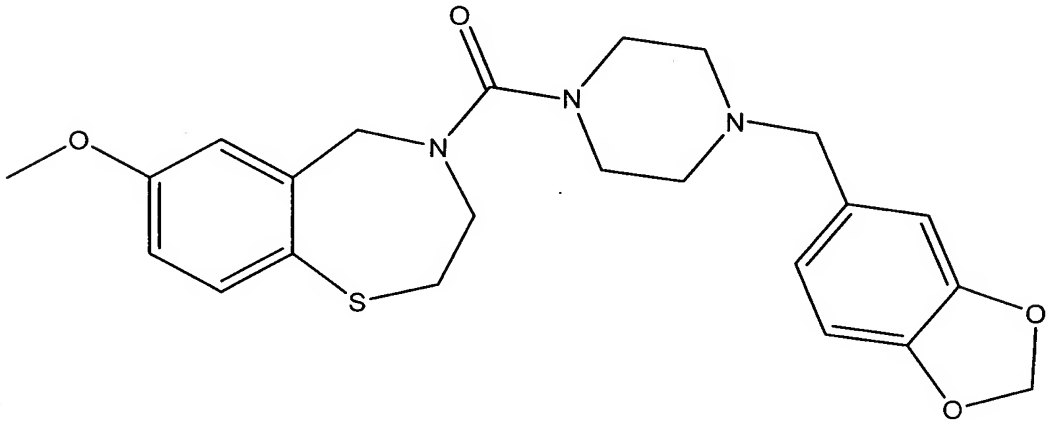

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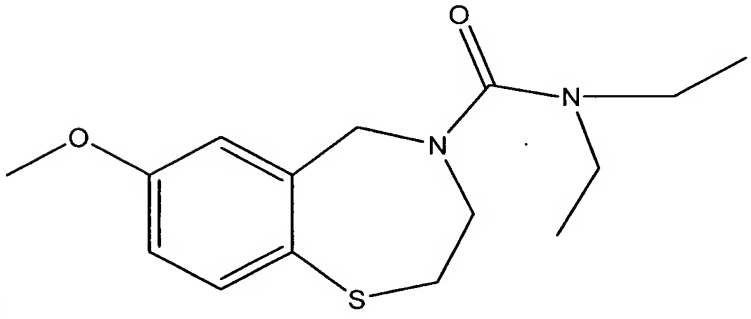

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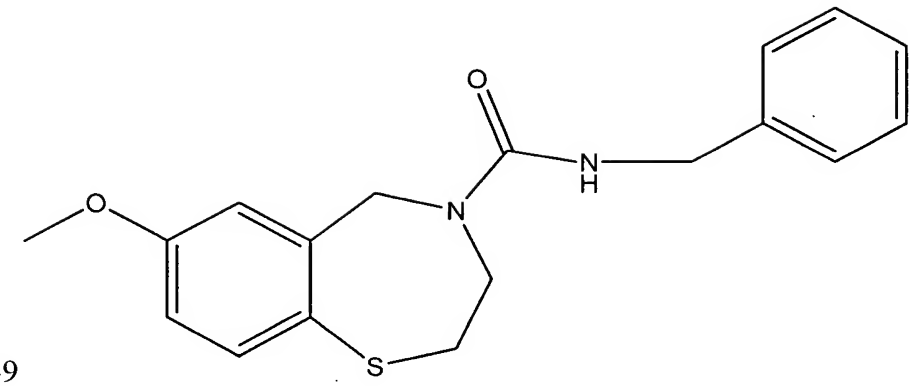

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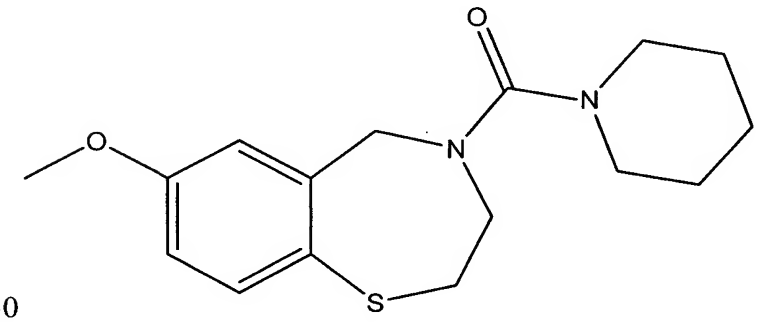

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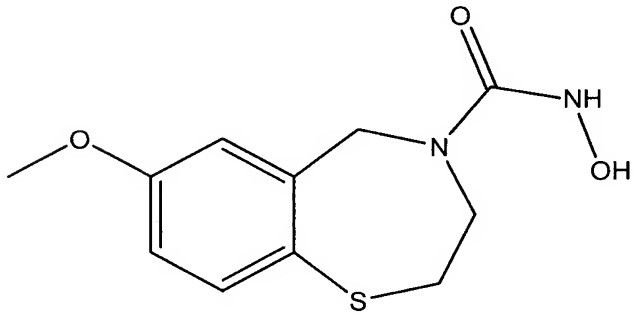

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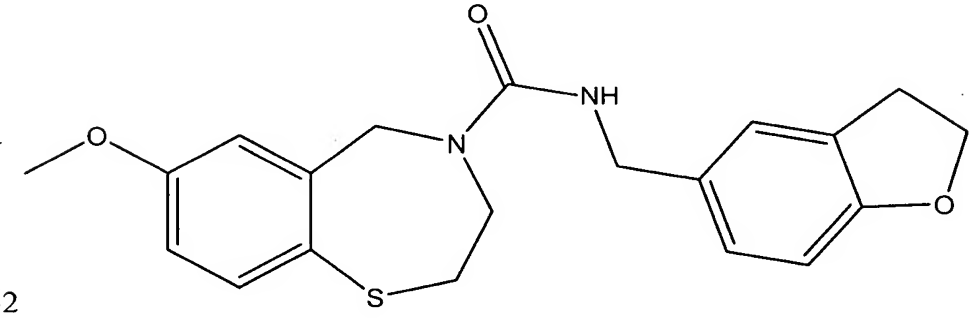

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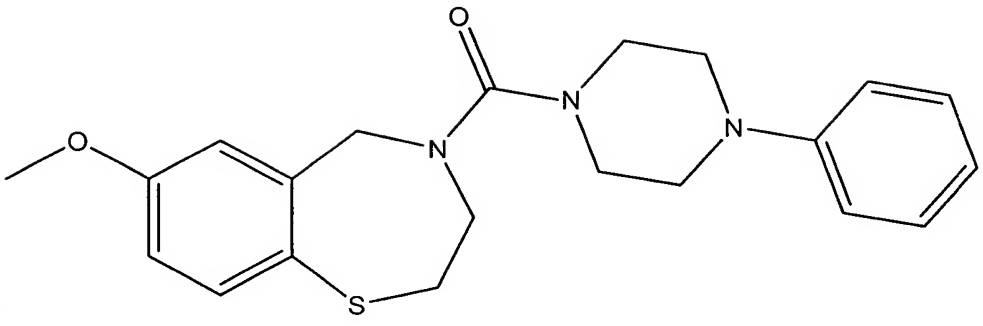

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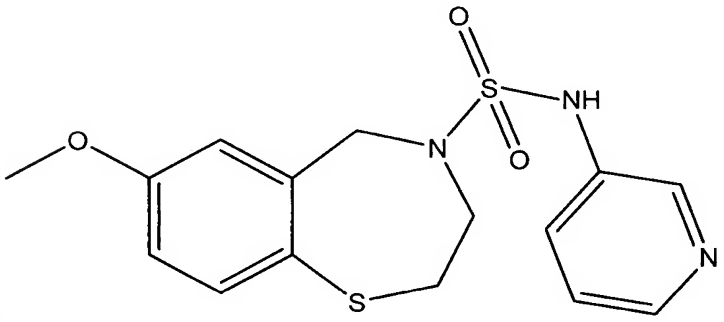
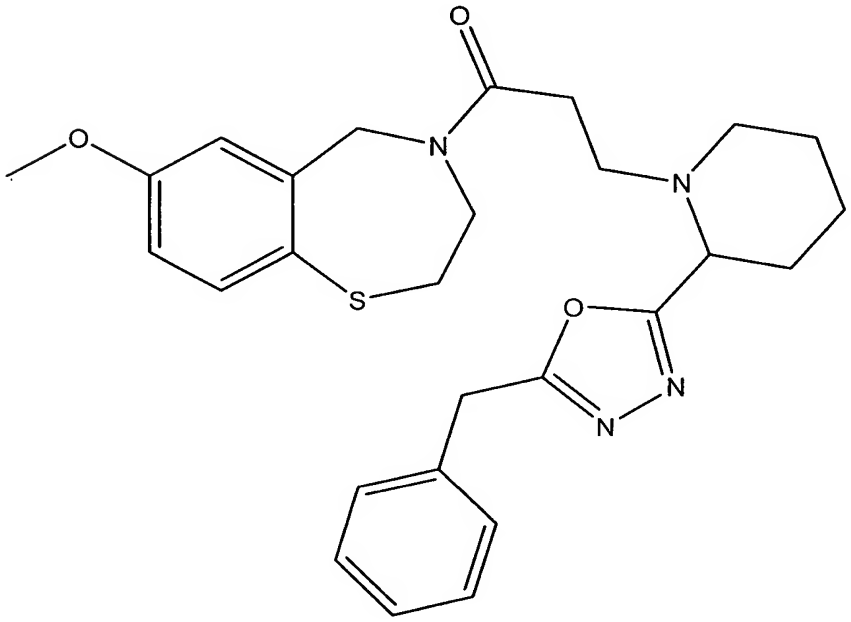


S54



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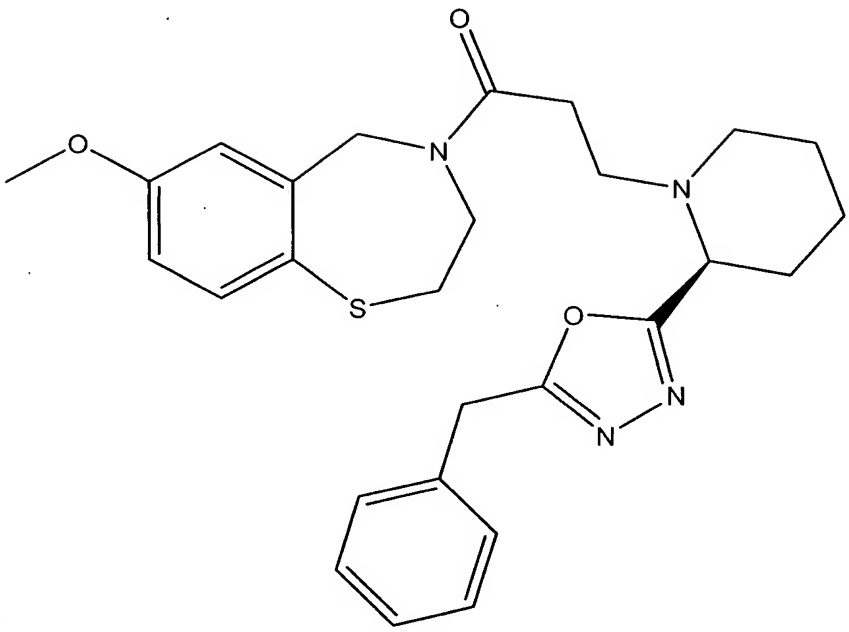

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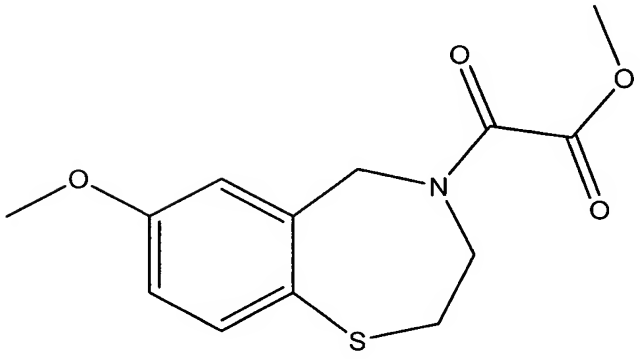

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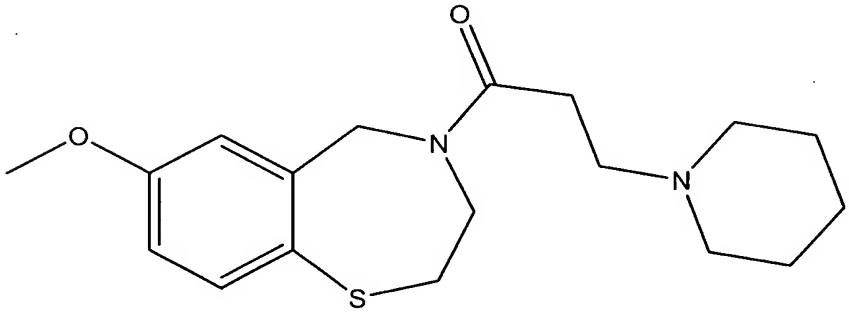

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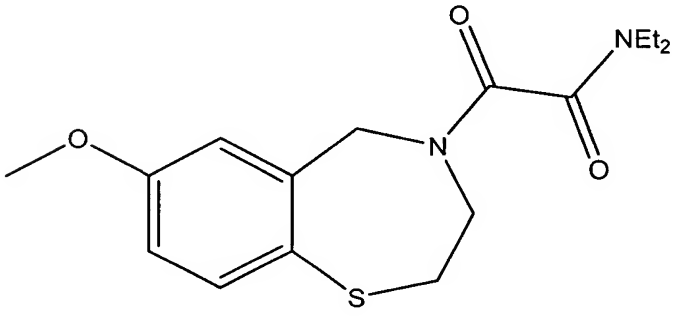

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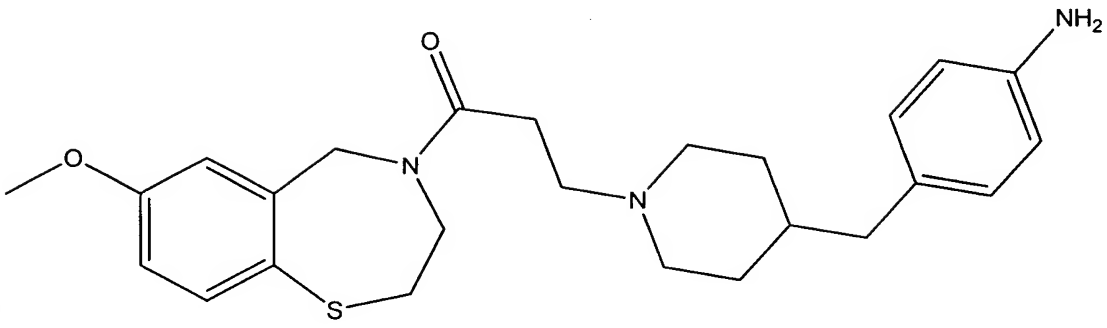

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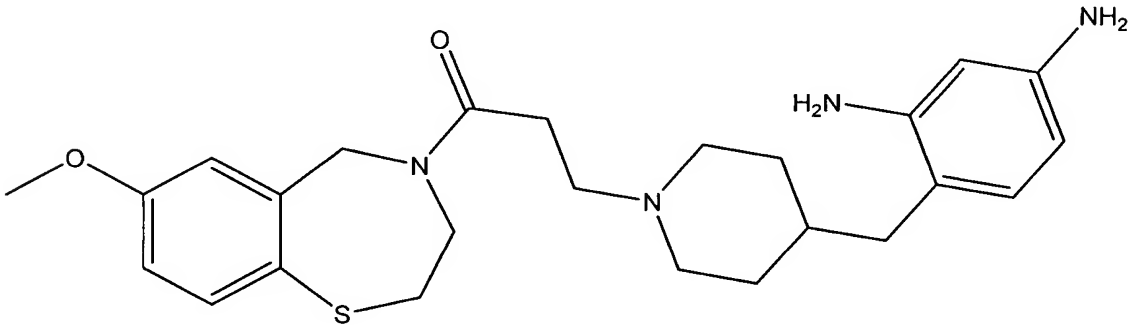

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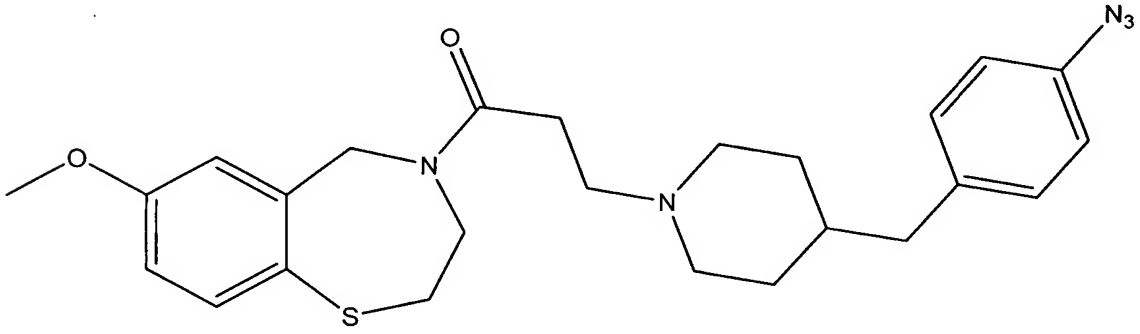

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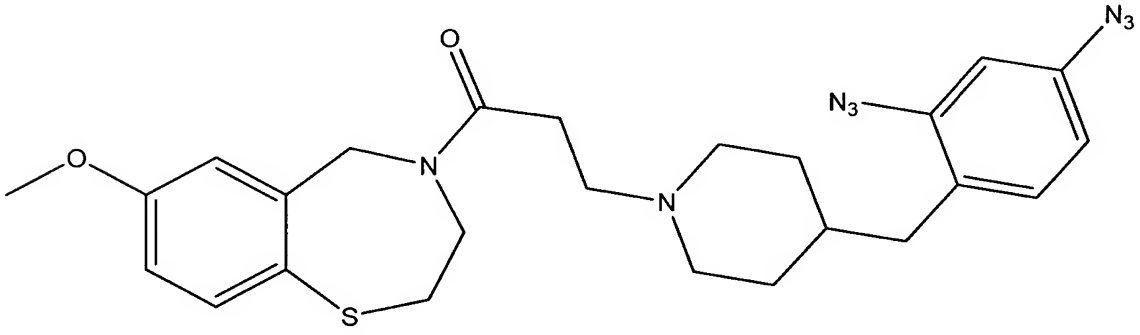

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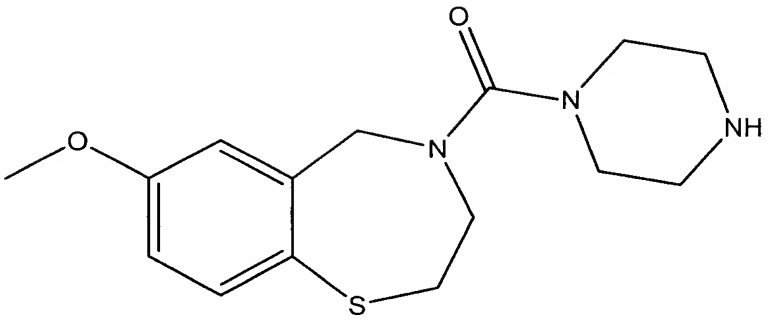

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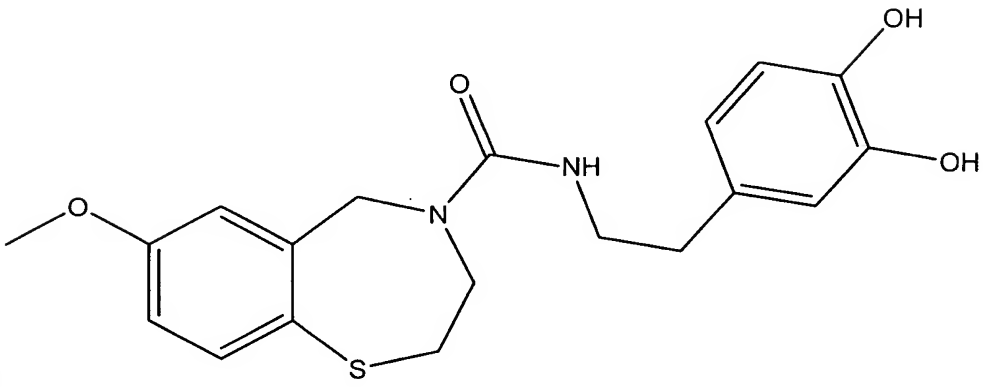

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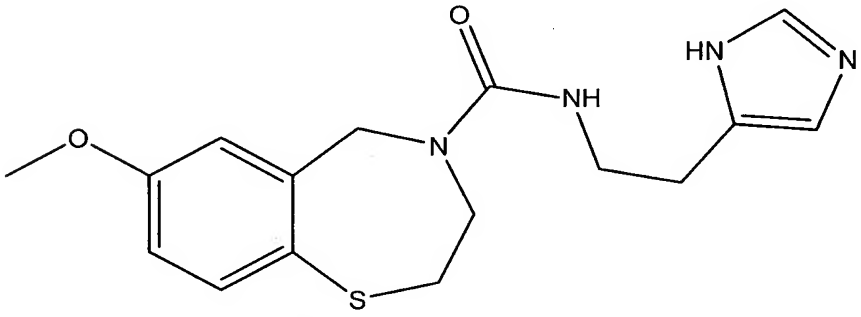

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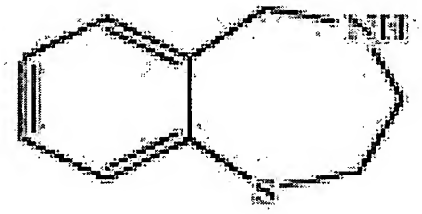

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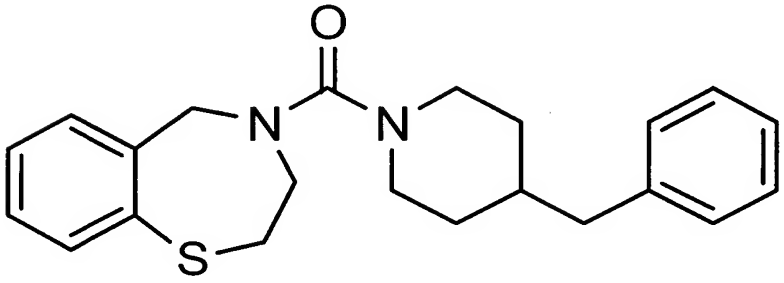

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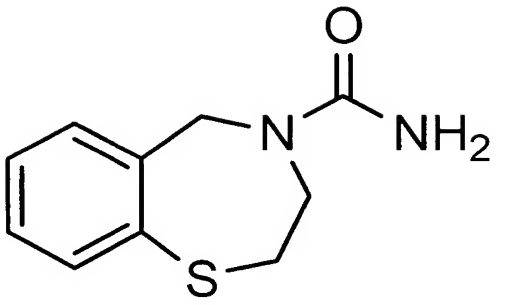

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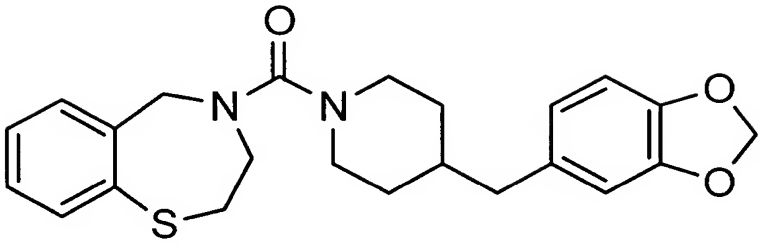

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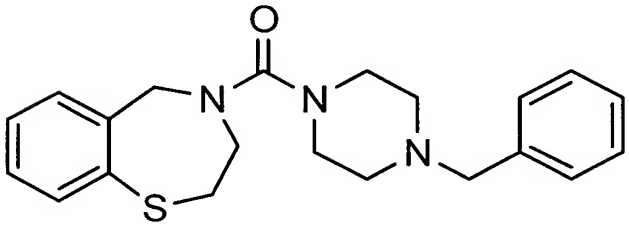

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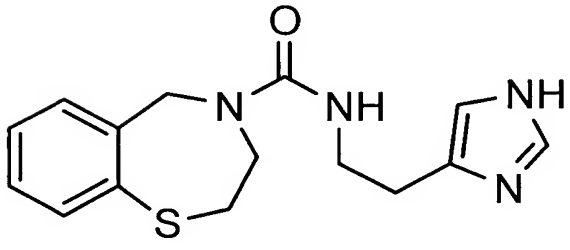

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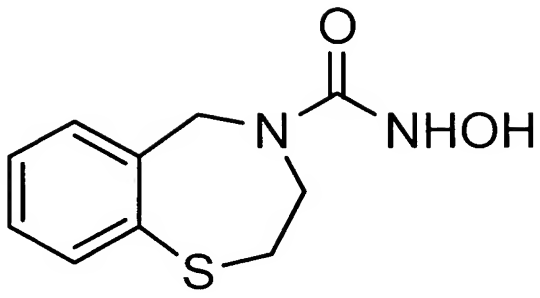

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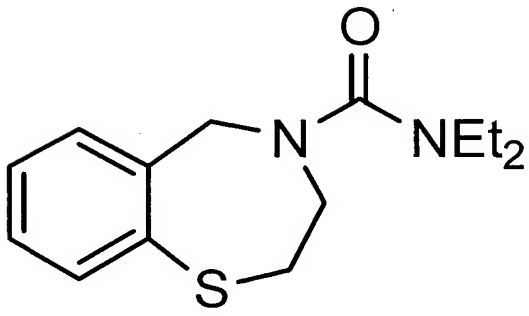

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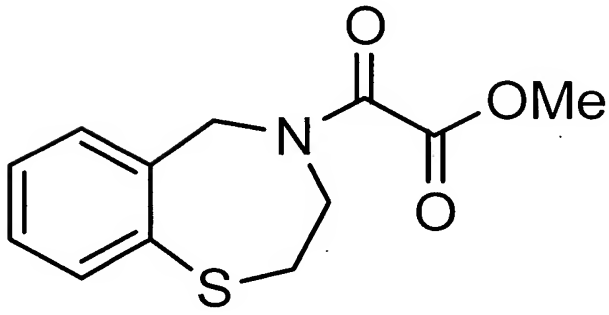

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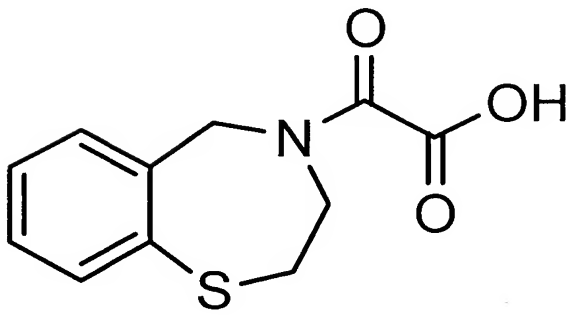

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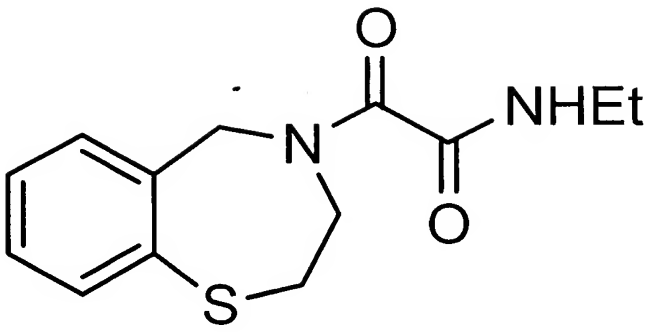

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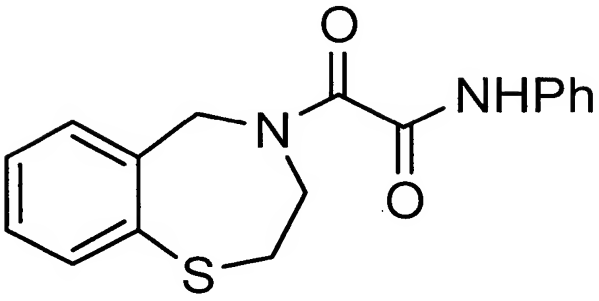

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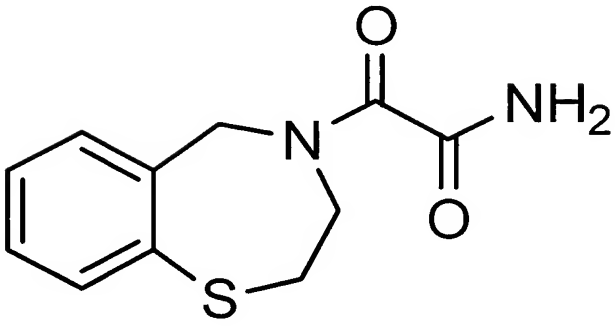

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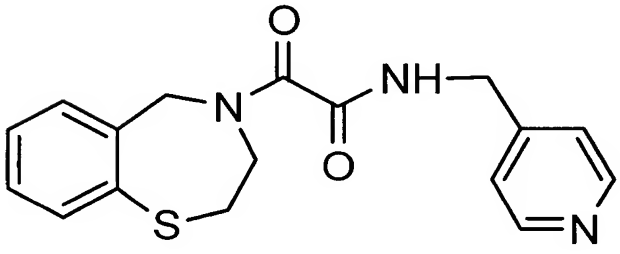

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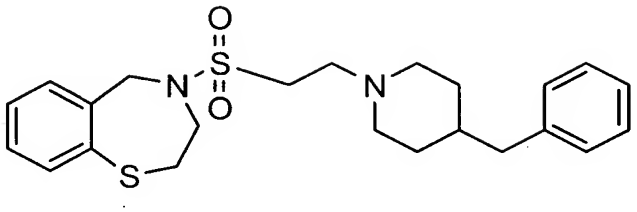

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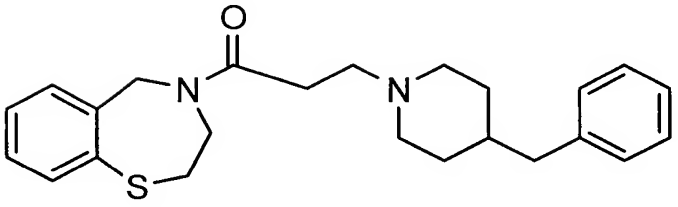

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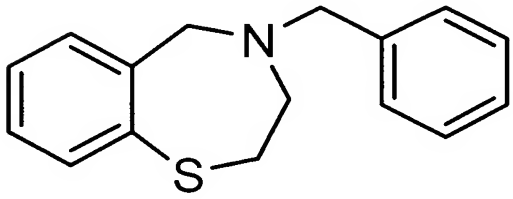

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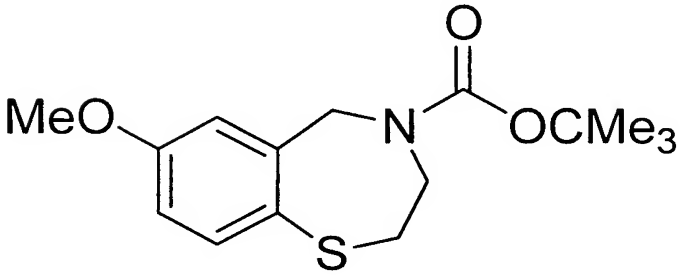

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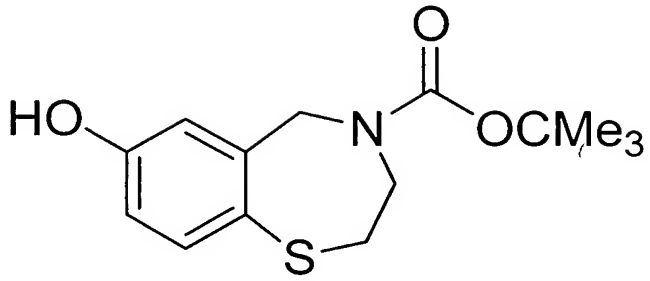

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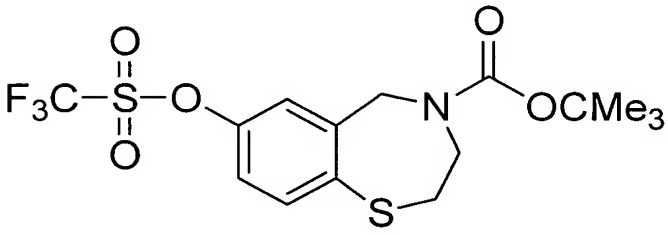

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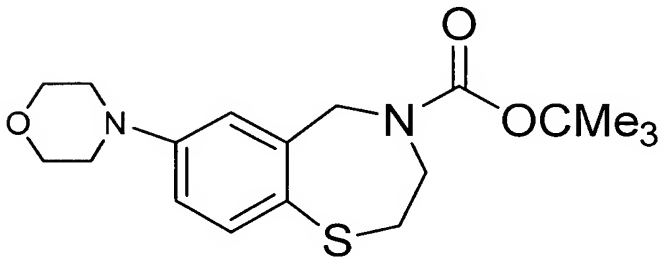

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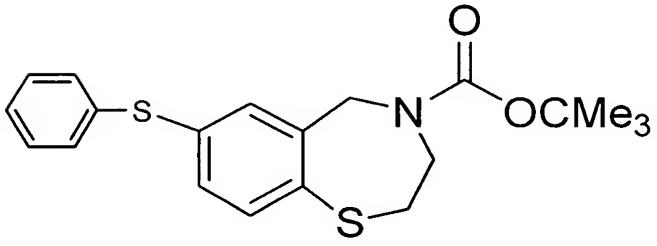

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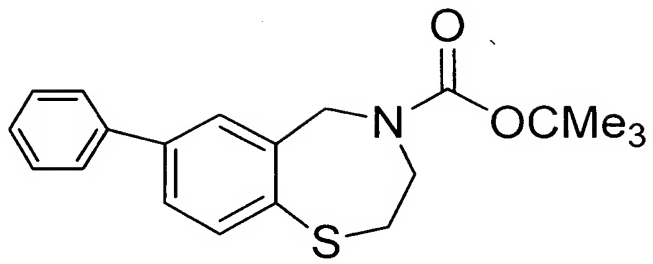

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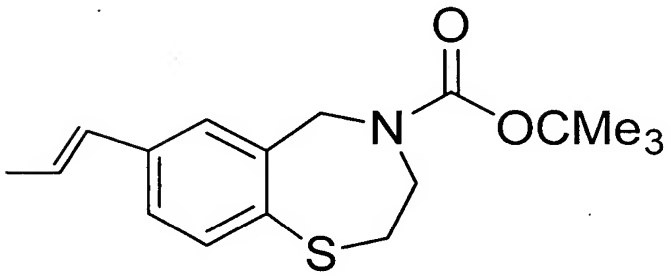

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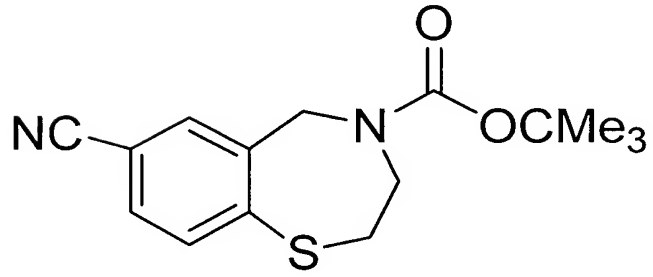

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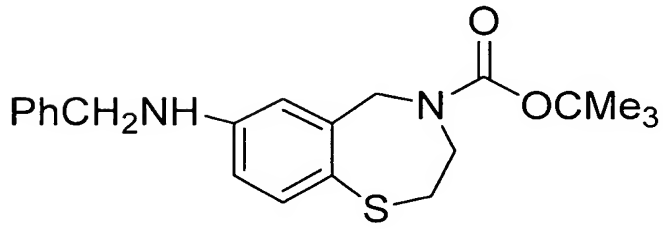

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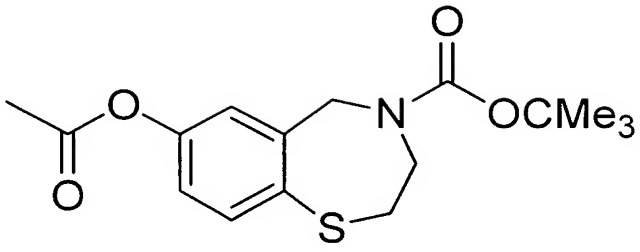

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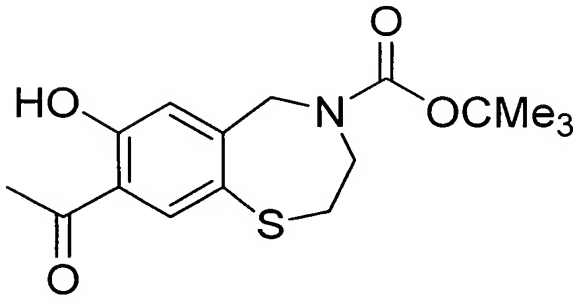

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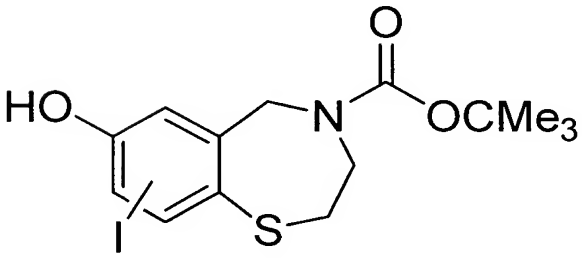

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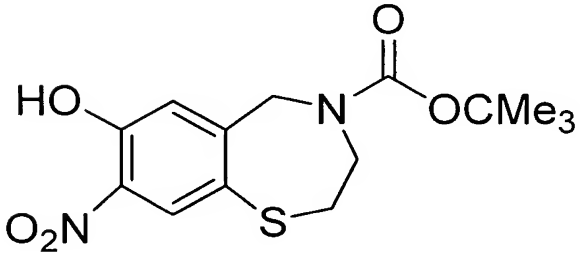

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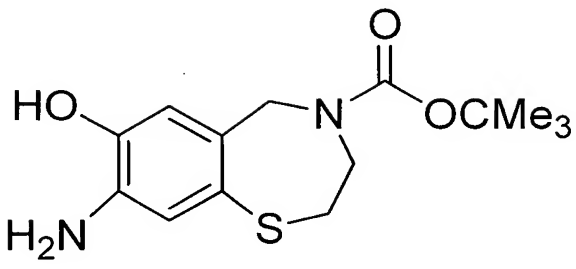

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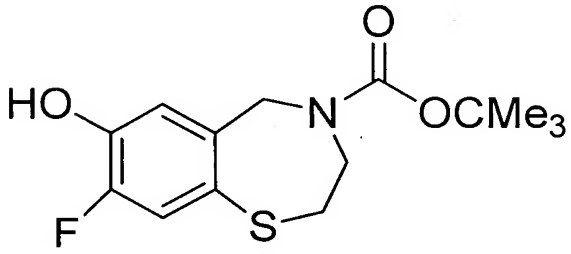

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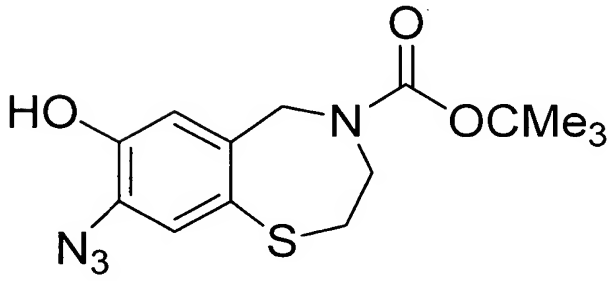

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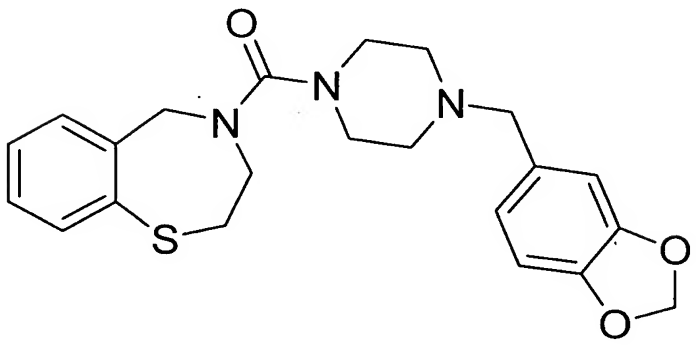

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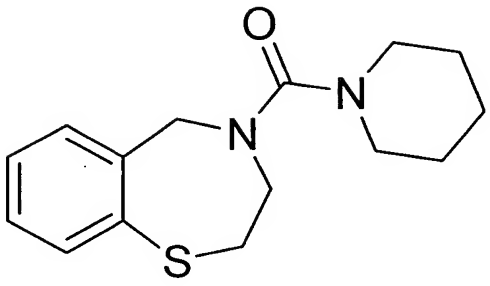

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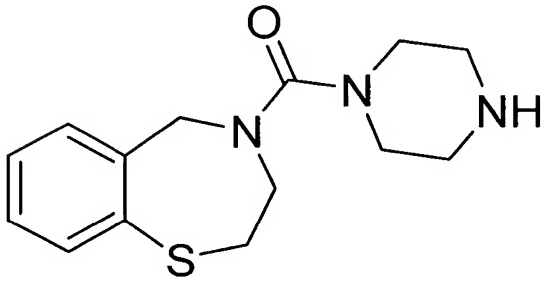

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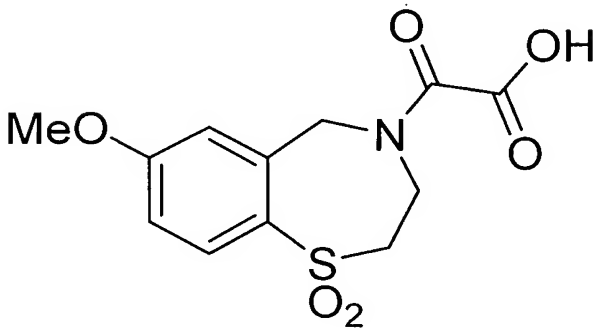

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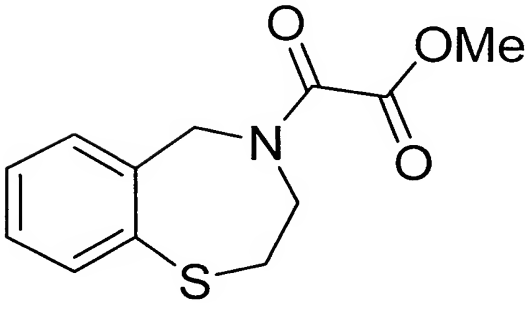

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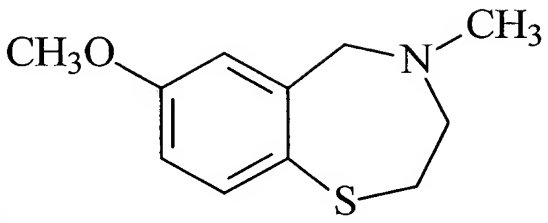

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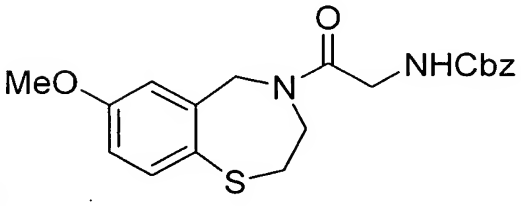

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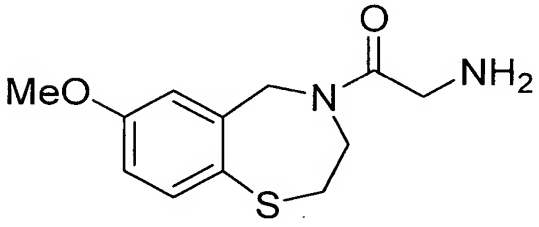

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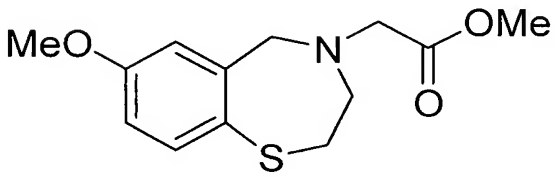

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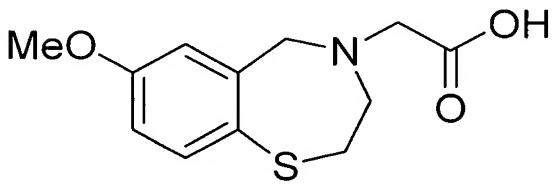

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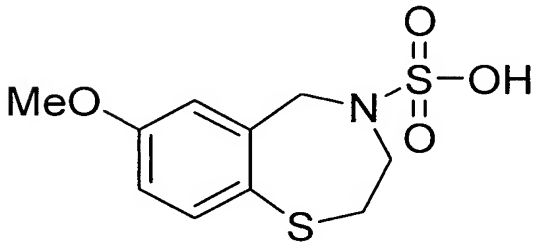

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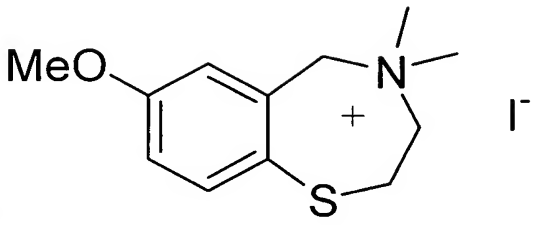

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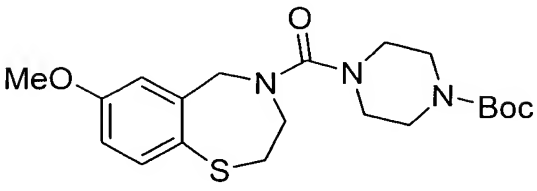

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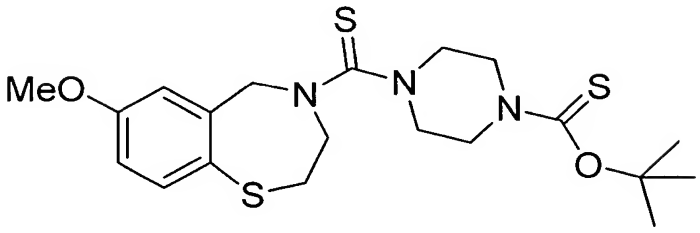

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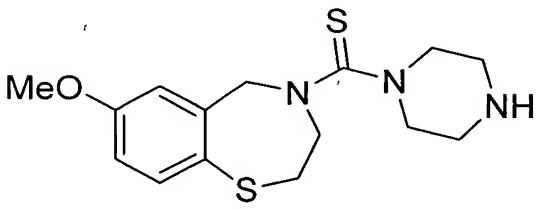

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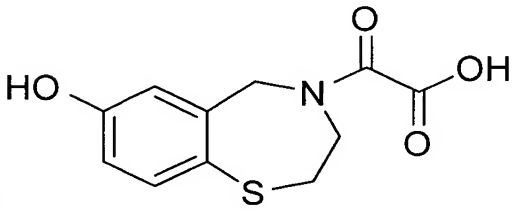

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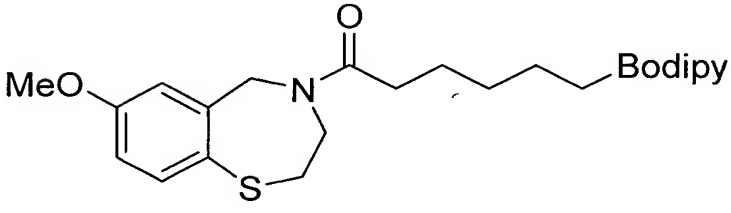

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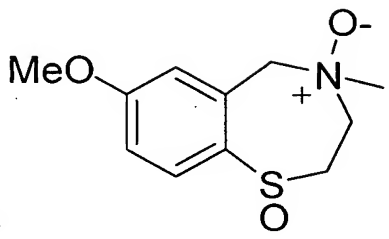

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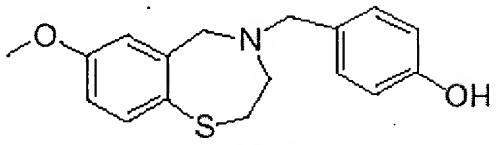
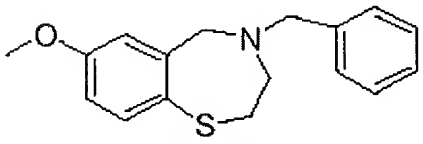


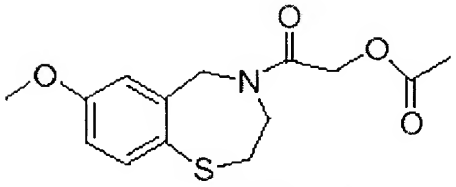
Arm120

S120



Arm121

S121



Arm122

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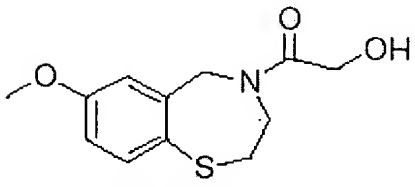


Arm123

S123

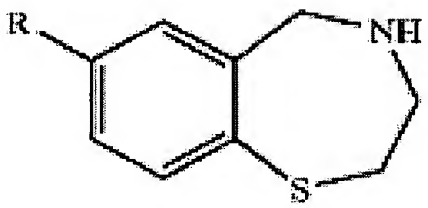
[00263] In one embodiment of the present invention, for compounds of Formula I, if R_2 is C=O(R_5) or SO_2R_7, then R is at positions 2, 3, or 5 on the benzene ring.

[00264] In another embodiment of the invention, for compounds of Formula I, if R_2 is $C=O(R_5)$ or SO_2R_7, then each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-N_3$, $-SO_3H$, acyl, alkyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, $-N_3$, -SH, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00265] In another embodiment of the invention, for compounds of Formula I, if R_2 is $C=O(R_5)$ or SO_2R_7, then there are at least two R groups attached to the benzene ring. Furthermore, there are at least two R groups attached to the benzene ring, and both R groups are attached at positions 2, 3, or 5 on the benzene ring. Still furthermore, each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-N_3$, $-SO_3H$, acyl, alkyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, $-N_3$, -SH, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00266] In another embodiment of the invention, for compounds of Formula I, if R_2 is $C=O(R_5)$, then R_5 is selected from the group consisting of $-NR_{16}$, $-(CH_2)_zNR_{15}R_{16}$, $NHNHR_{16}$, NHOH, $-OR_{15}$, $CONH_2NHR_{16}$, $CONR_{16}$, CH_2X, acyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, $-N_3$, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00267] In another embodiment, the present invention provides use of compounds of Formula II:



wherein R=OR', SR', NR', alkyl, or halide and R' = alkyl, aryl, or H, and wherein R can be at position 2, 3, 4, or 5. Formula II is discussed also in co-pending application 10/680,988, the disclosure of which is incorporated herein in its entirety by reference.

Routes of Activity

[00268] The compounds of the invention reduce the open probability of RyR by increasing the affinity of FKBP12 (calstabin1) and FKBP12.6 (calstabin2) for, respectively PKA-phosphorylated RyR1 and PKA-phosphorylated RyR2. Moreover, the compounds of Formula I normalize gating of mutant RyR channels, including CPVT-associated mutant RyR2 channels, by increasing FKBP12 (calstabin1) and FKBP12.6 (calstabin2) binding affinity. Therefore, the compounds of the invention prevent disorders and conditions involving modulation of the RyR receptors, particularly the RyR1 and RyR2 receptors. Examples of such disorders and conditions include, without limitation, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include and exercise-induced irregular heartbeat disorders and diseases include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation; atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss. The compounds of the invention treat these disorders and conditions by increasing FKBP12 (calstabin1)-RyR1 binding affinity and increasing FKBP12.6 (calstabin2)-RyR2 binding affinity.

[00269] In accordance with the foregoing, the present invention provides a method for limiting or preventing a decrease in the level of RyR-bound FKBP (calstabin) in cells of a subject. As used herein, "RyR" includes RyR1, RyR2, and RyR3. Additionally, FKBP includes both FKBP12 (calstabin1) and FKBP12.6 (calstabin2). "RyR-bound FKBP" therefore refers to RyR1-bound FKBP12 (calstabin1), RyR2-bound FKBP12.6 (calstabin2), and RyR3-bound FKBP12 (calstabin1).

[00270] As used herein, "RyR" also includes an "RyR protein" and an "RyR analogue." An "RyR analogue" is a functional variant of the RyR protein, having RyR biological activity, that has 60% or greater amino-acid-sequence homology with the RyR protein. The RyR of the present invention are unphosphorylated, phosphorylated (*e.g.*, by PKA), or hyperphosphorylated (*e.g.*, by PKA). As further used herein, the term "RyR biological activity" refers to the activity of a protein or peptide that demonstrates an ability to associate physically with, or bind with, FKBP12 (calstabin1) in the case of RyR1 and RyR3, and FKBP12.6 (calstabin2) in the case of RyR2 (*i.e.*, binding of approximately two fold or, approximately five fold, above the background binding of a negative control), under the conditions of the assays described herein.

[00271] As used herein, "FKBP" includes both an "FKBP protein" and an "FKBP analogue," whether it be FKBP12 (calstabin1) or FKBP12.6 (calstabin2). Unless otherwise indicated herein, "protein" shall include a protein, protein domain, polypeptide, or peptide, and any fragment thereof. An "FKBP analogue" is a functional variant of the FKBP protein, having FKBP biological activity, that has 60% or greater amino-acid-sequence homology with the FKBP protein, whether it be FKBP12 (calstabin1) or FKBP12.6 (calstabin2). As further used herein, the term "FKBP biological activity" refers to the activity of a protein or peptide that demonstrates an ability to associate physically with, or bind with, unphosphorylated or non-hyperphosphorylated RyR2 (*i.e.*, binding of approximately two fold, or approximately five fold, above the background binding of a negative control), under the conditions of the assays described herein.

[00272] FKBP binds to the RyR channel, one molecule per RyR subunit. Accordingly, as used herein, the term "RyR-bound FKBP" includes a molecule of an FKBP12 (calstabin1) protein that is bound to an RyR1 protein subunit or a tetramer of FKBP12 that is in association with a tetramer of RyR1, a molecule of FKBP12.6 (calstabin2) protein that is

bound to an RyR2 protein subunit or a tetramer of FKBP12.6 that is in association with a tetramer of RyR2, and a molecule of an FKBP12 (calstabin1) protein that is bound to an RyR3 protein subunit or a tetramer of FKBP12 that is in association with a tetramer of RyR3. Therefore, "RyR-bound FKBP" refers to "RyR1-bound FKBP12," "RyR2-bound FKBP12.6," and "RyR3-bound FKBP12."

[00273] In accordance with the method of the present invention, a "decrease" or "disorder" in the level of RyR-bound FKBP in cells of a subject refers to a detectable decrease, diminution or reduction in the level of RyR-bound FKBP in cells of the subject. Such a decrease is limited or prevented in cells of a subject when the decrease is in any way halted, hindered, impeded, obstructed or reduced by the administration of compounds of the invention, such that the level of RyR-bound FKBP in cells of the subject is higher than it would otherwise be in the absence of the administered compound.

[00274] The level of RyR-bound FKBP in a subject is detected by standard assays and techniques, including those readily determined from the known art (*e.g.*, immunological techniques, hybridization analysis, immunoprecipitation, Western-blot analysis, fluorescence imaging techniques and/or radiation detection, etc.), as well as any assays and detection methods disclosed herein. For example, protein is isolated and purified from cells of a subject using standard methods known in the art, including, without limitation, extraction from the cells (*e.g.*, with a detergent that solubilizes the protein) where necessary, followed by affinity purification on a column, chromatography (*e.g.*, FTLC and HPLC), immunoprecipitation (with an antibody), and precipitation (*e.g.*, with isopropanol and a reagent such as Trizol). Isolation and purification of the protein is followed by electrophoresis (*e.g.*, on an SDS-polyacrylamide gel). A decrease in the level of RyR-bound FKBP in a subject, or the limiting or prevention thereof, is determined by comparing the amount of RyR-bound FKBP detected prior to the administration of a compound of the invention I (in accordance with methods described below) with the amount detected a suitable time after administration of the compound.

[00275] A decrease in the level of RyR-bound FKBP in cells of a subject is limited or prevented, for example, by inhibiting dissociation of FKBP and RyR in cells of the subject; by increasing binding between FKBP and RyR in cells of the subject; or by stabilizing the RyR-FKBP complex in cells of a subject. Additionally, a decrease in the level of RyR-bound

FKBP in cells of a subject is limited or prevented by directly decreasing the level of phosphorylated RyR in cells of the subject or by indirectly decreasing the level of phosphorylated RyR in the cells (*e.g.*, by targeting an enzyme (such as PKA) or another endogenous molecule that regulates or modulates the functions or levels of phosphorylated RyR in the cells). In one embodiment, the level of phosphorylated RyR in the cells is decreased by at least 10% in the method of the present invention. In another embodiment, the level of phosphorylated RyR is decreased by at least 20%.

[00276] The subject of the present invention are *in vitro* and *in vivo* systems, including, without limitation, isolated or cultured cells or tissues, non-cell *in vitro* assay systems and an animal (*e.g.*, an amphibian, a bird, a fish, a mammal, a marsupial, a human, a domestic animal (such as a cat, dog, monkey, horse, mouse or rat) or a commercial animal (such as a cow or pig)).

[00277] The cells of a subject include striated muscle cells. A striated muscle is a muscle in which the repeating units (sarcomeres) of the contractile myofibrils are arranged in registry throughout the cell, resulting in transverse or oblique striations that are observed at the level of a light microscope. Examples of striated muscle cells include, without limitation, voluntary (skeletal) muscle cells and cardiac muscle cells. In one embodiment, the cell used in the method of the present invention is a human cardiac muscle cell. As used herein, the term "cardiac muscle cell" includes cardiac muscle fibers, such as those found in the myocardium of the heart. Cardiac muscle fibers are composed of chains of contiguous heart-muscle cells, or cardiomyocytes, joined end to end at intercalated disks. These disks possess two kinds of cell junctions: expanded desmosomes extending along their transverse portions, and gap junctions, the largest of which lie along their longitudinal portions.

[00278] A decrease in the level of RyR-bound FKBP is limited or prevented in cells of a subject by administering the compounds of the invention to the subject; this would also permit contact between cells of the subject and the compounds of the invention. The compounds of the invention are modulators of calcium-ion channels. In addition to regulating Ca^{2+} levels in myocardial cells, the compounds of the invention modulate the Na^{+} current and the inward-rectifier K^{+} current in cells, such as guinea pig ventricular cells, and inhibits the delayed-rectifier K^{+} current in cells, such as guinea pig atrial cells.

Pharmaceutical Composition

[00279] The compounds of the invention are formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration *in vivo*. According to another aspect, the present invention provides a pharmaceutical composition comprising compounds of the invention in admixture with a pharmaceutically acceptable diluent and/or carrier. The pharmaceutically-acceptable carrier must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. The pharmaceutically-acceptable carrier employed herein is selected from various organic or inorganic materials that are used as materials for pharmaceutical formulations and which are incorporated as analgesic agents, buffers, binders, disintegrants, diluents, emulsifiers, excipients, extenders, glidants, solubilizers, stabilizers, suspending agents, tonicity agents, vehicles and viscosity-increasing agents. If necessary, pharmaceutical additives, such as aromatics, colorants, flavor-improving agents, preservatives, and sweeteners, are also added. Examples of acceptable pharmaceutical carriers include carboxymethyl cellulose, crystalline cellulose, glycerin, gum arabic, lactose, magnesium stearate, methyl cellulose, powders, saline, sodium alginate, sucrose, starch, talc and water, among others.

[00280] The pharmaceutical formulations of the present invention are prepared by methods well-known in the pharmaceutical arts. For example, the compounds of the invention are brought into association with a carrier and/or diluent, as a suspension or solution. Optionally, one or more accessory ingredients (*e.g.*, buffers, flavoring agents, surface active agents, and the like) also are added. The choice of carrier is determined by the solubility and chemical nature of the compounds, chosen route of administration and standard pharmaceutical practice.

[00281] The compounds of the invention are administered to a subject by contacting target cells (*e.g.*, cardiac muscle cells) *in vivo* in the subject with the compounds. The compounds of the invention are contacted with (*e.g.*, introduced into) cells of the subject using known techniques utilized for the introduction and administration of proteins, nucleic acids and other drugs. Examples of methods for contacting the cells with (*i.e.*, treating the cells with) the compounds of the invention include, without limitation, absorption, electroporation, immersion, injection, introduction, liposome delivery, transfection, transfusion, vectors and other drug-delivery vehicles and methods. When the target cells are localized to a particular portion of a subject, it is desirable to introduce the compounds for the

invention directly to the cells, by injection or by some other means (*e.g.*, by introducing the compounds into the blood or another body fluid). The target cells are contained in tissue of a subject and are detected by standard detection methods readily determined from the known art, examples of which include, without limitation, immunological techniques (*e.g.*, immunohistochemical staining), fluorescence imaging techniques, and microscopic techniques.

[00282] Additionally, the compounds of the present invention are administered to a human or animal subject by known procedures including, without limitation, oral administration, sublingual or buccal administration, parenteral administration, transdermal administration, via inhalation or intranasally, vaginally, rectally, and intramuscularly. The compounds of the invention are administered parenterally, by epifascial, intracapsular, intracranial, intracutaneous, intrathecal, intramuscular, intraorbital, intraperitoneal, intraspinal, intrasternal, intravascular, intravenous, parenchymatous, subcutaneous or sublingual injection, or by way of catheter. In one embodiment, the agent is adiminstered to the subject by way of delivery to the subject's muscles including, but not limited to, the subject's cardiac muscles. In an embodiment, the agent is administered to the subject by way of targeted delivery to cardiac muscle cells *via* a catheter inserted into the subject's heart. In other embodiments, the agent is administered via a subcutaneous pump.

[00283] For oral administration, a formulation of the compounds of the invention are presented as capsules, tablets, powders, granules or as a suspension or solution. The formulation has conventional additives, such as lactose, mannitol, corn starch or potato starch. The formulation also is presented with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins. Additionally, the formulation is presented with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose. The formulation also is presented with dibasic calcium phosphate anhydrous or sodium starch glycolate. Finally, the formulation is presented with lubricants, such as talc or magnesium stearate.

[00284] For parenteral administration (*i.e.*, administration by injection through a route other than the alimentary canal), the compounds of the invention are combined with a sterile aqueous solution that is isotonic with the blood of the subject. Such a formulation is prepared by dissolving a solid active ingredient in water containing physiologically-compatible

substances, such as sodium chloride, glycine and the like, and having a buffered pH compatible with physiological conditions, so as to produce an aqueous solution, then rendering said solution sterile. The formulation is presented in unit or multi-dose containers, such as sealed ampoules or vials. The formulation is delivered by any mode of injection, including, without limitation, epifascial, intracapsular, intracranial, intracutaneous, intrathecal, intramuscular, intraorbital, intraperitoneal, intraspinal, intrasternal, intravascular, intravenous, parenchymatous, subcutaneous, or sublingual or by way of catheter into the subject's heart.

[00285] For transdermal administration, the compounds of the invention are combined with skin penetration enhancers, such as propylene glycol, polyethylene glycol, isopropanol, ethanol, oleic acid, *N*-methylpyrrolidone and the like, which increase the permeability of the skin to the compounds of the invention and permit the compounds to penetrate through the skin and into the bloodstream. The compounds of the invention /enhancer composition also are further combined with a polymeric substance, such as ethylcellulose, hydroxypropyl cellulose, ethylene/vinylacetate, polyvinyl pyrrolidone, and the like, to provide the composition in gel form, which are dissolved in a solvent, such as methylene chloride, evaporated to the desired viscosity and then applied to backing material to provide a patch.

[00286] In some embodiments, the composition is in unit dose form such as a tablet, capsule or single-dose vial. Suitable unit doses, *i.e.*, therapeutically effective amounts, can be determined during clinical trials designed appropriately for each of the conditions for which administration of a chosen compound is indicated and will, of course, vary depending on the desired clinical endpoint. The present invention also provides articles of manufacture for treating and preventing disorders, such as cardiac disorders, in a subject. The articles of manufacture comprise a pharmaceutical composition of one or more of the compounds of the invention as described herein. The articles of manufacture are packaged with indications for various disorders that the pharmaceutical compositions are capable of treating and/or preventing. For example, the articles of manufacture comprise a unit dose of a compound disclosed herein that is capable of treating or preventing a muscular disorder, and an indication that the unit dose is capable of treating or preventing a certain disorder, for example an arrhythmia.

[00287] In accordance with a method of the present invention, the compounds of the invention are administered to the subject (or are contacted with cells of the subject) in an amount effective to limit or prevent a decrease in the level of RyR-bound FKBP in the subject, particularly in cells of the subject. This amount is readily determined by the skilled artisan, based upon known procedures, including analysis of titration curves established *in vivo* and methods and assays disclosed herein. A suitable amount of the compounds of the invention effective to limit or prevent a decrease in the level of RyR-bound FKBP in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml. In an embodiment, the amount of compounds from the invention ranges from about 10 mg/kg/day to about 20 mg/kg/day.

Uses

[00288] The present invention provides a new range of therapeutic treatments for patients with various disorders involving modulation of the RyR receptors, particularly skeletal muscular disorders (RyR1). The present application also provides new therapeutic treatment strategies for patients with muscle fatigue resulting from various disease states and disordres that are assocdiated with RyR, including, but not limited to cardiac (RyR2) disorders, and/or cognitive (RyR3) disorders.

[00289] In one embodiment of the present invention, the subject has not yet developed symptoms of muscle fatigue, (*e.g.*, exercise-induced muscle fatigue). In another embodiment of the present invention, the subject is in need of treatment for a disorder associated with muscle fatigue, including skeletal muscular disorders.

[00290] Various disorders that the compounds of the invention treat or prevent include, but are not limited to, cardiac disorders and diseases, skeletal muscular disorders and diseases, cognitive disorders and diseases, malignant hyperthermia, diabetes, and sudden infant death syndrome. Cardiac disorder and diseases include, but are not limited to, irregular heartbeat disorders and diseases; exercise-induced irregular heartbeat disorders and diseases; sudden cardiac death; exercise-induced sudden cardiac death; congestive heart failure; chronic obstructive pulmonary disease; and high blood pressure. Irregular heartbeat disorders and diseases include and exercise-induced irregular heartbeat disorders and diseases include, but are not limited to, atrial and ventricular arrhythmia; atrial and ventricular fibrillation;

atrial and ventricular tachyarrhythmia; atrial and ventricular tachycardia; catecholaminergic polymorphic ventricular tachycardia (CPVT); and exercise-induced variants thereof. Skeletal muscular disorder and diseases include, but are not limited to, skeletal muscle fatigue, exercise-induced skeletal muscle fatigue, muscular dystrophy, bladder disorders, and incontinence. Cognitive disorders and diseases include, but are not limited to, Alzheimer's Disease, forms of memory loss, and age-dependent memory loss. One skilled in the art will recognize still other diseases, including but not limited to muscular and cardiac disorders, that the compounds of the invention are useful to treat, in accordance with the information provided herein.

[00291] The amount of compounds of the invention effective to limit or prevent a decrease in the level of RyR1-bound FKBP12 in the subject is an amount effective to prevent muscle fatigue in the subject. This amount is readily determined by the skilled artisan, based upon known procedures, including clinical trials, and methods disclosed herein.

[00292] Because of its ability to stabilize RyR-bound FKBP and maintain and restore balance in the context of dynamic PKA phosphorylation and dephosphorylation of RyR, the compounds of the invention are also useful in treating a subject who has already experienced clinical symptoms of these various disorders. For example, if the symptoms of the disorder are observed in the subject early enough, the compounds of the invention are effective in limiting or preventing a further decrease in the level of RyR-bound FKBP in the subject.

[00293] Additionally, the subject of the present invention can be a candidate for muscle fatigue disorder, including but not limited to any chronic disorder associated with muscle fatigue, or stress or exercise-induced muscle fatigue.

[00294] Accordingly, in still another embodiment of the present invention, the subject has been exercising, or is currently exercising, and has developed an exercise-induced disorder. In this case, the amount of the compounds of the invention effective to limit or prevent a decrease in the level of RyR-bound FKBP in the subject is an amount of compound effective to treat the exercise-induced disorder in the subject. As used herein, an amount of compounds of the invention "effective to treat an exercise-induced disorder" includes an amount of compound of the invention effective to alleviate or ameliorate the clinical impairment or symptoms of the exercise-induced disorder characterized by muscle fatigue. The amount of compounds of the invention effective to treat an exercise-induced disorder in a

subject will vary depending upon the particular factors of each case, including the type of exercise-induced disorder, the subject's weight, the severity of the subject's condition, and the mode of administration of the compounds of the invention. This amount is readily determined by the skilled artisan, based upon known procedures, including clinical trials, and methods disclosed herein. In an embodiment, the compounds of the invention treat exercise-induced disorders in the subject.

[00295] The present invention further provides a method for treating muscle fatigue associated with exercise-induced disorders and conditions in a subject. The method comprises administering the compounds of the invention to the subject in an amount effective to treat the exercise-induced disorder in the subject. A suitable amount of the compounds of the invention effective to treat, for example, exercise-induced cardiac arrhythmia in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml. The present invention also provides a method for preventing an exercise-induced disorder in a subject. The method comprises administering the compounds of the invention to the subject in an amount effective to prevent the exercise-induced disorder in the subject. A suitable amount of the compounds of the invention effective to prevent the exercise-induced disorder in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml. Additionally, the present invention provides a method for preventing exercise-induced disorders in a subject. The method comprises administering the compounds of the invention to the subject in an amount effective to prevent an exercise-induced disorder in the subject. A suitable amount of the compounds of the invention effective to prevent an exercise-induced disorder in the subject ranges from about 5 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml.

[00296] The compounds of the invention can be used alone, in combination with each other, or in combination with other agents that have cardiovascular activity including, but not limited to, diuretics, anticoagulants, antiplatelet agents, antiarrhythmics, inotropic agents, chronotropic agents, α and β blockers, angiotensin inhibitors and vasodilators. Further, such combinations of the compounds of the present invention and other cardiovascular agents are administered separately or in conjunction. In addition, the administration of one element of

the combination is prior to, concurrent to or subsequent to the administration of other agent(s).

[00297] In view of the foregoing methods, the present invention also provides use of the compounds of the invention in a method for limiting or preventing a decrease in the level of RyR-bound FKBP in a subject who is a candidate for a disorder. The present invention also provides use of the compounds of the invention in a method for treating or preventing a muscular disorder in a subject. Furthermore, the present invention provides use of the compounds of the invention in a method for preventing treating or preventing exercise-induced muscular disorders in a subject.

[00298] Intracellular Ca^{2+} leak is proposed as a principal mediator of depressed muscle performance and dystrophic muscle remodeling. Muscular dystrophies are heterogeneous hereditary diseases characterized by weakness and progressive muscle wasting. Of all forms of muscular dystrophies involving the dystrophin-associated protein complex (referred to as dystrophinopathies), Duchenne muscular dystrophy (DMD) is one of the most frequent genetic diseases (X-linked; 1 in 3,500 boys) with death usually occurring before age 30 by respiratory and/or cardiac failure in high numbers of patients. Becker muscular dystrophy (BMD) represents a milder form of the disease associated with a reduction in the amount or expression of a truncated form of the dystrophin protein whereas Duchenne patients have been characterized by complete absence or very low levels of dystrophin. Duchenne and Becker's muscular dystrophy (DMD/BMD) are caused by mutations in the gene encoding the 427-kDa cytoskeletal protein dystrophin. However, with increasing age in BMD cardiac symptoms are more common than in DMD patients and do not correlate with skeletal muscle symptoms. Since genetic screening will not eliminative DMD due to a high incidence of sporadic cases, an effective therapy is highly desirable. DMD/BMD have been consistently associated with disturbed intracellular calcium metabolism. Because alterations of intracellular Ca^{2+} concentrations in DMD myofibers are believed to represent a central pathogenic mechanism, development of a therapeutic intervention that prevents intracellular Ca^{2+} abnormalities as a cause of skeletal muscle degeneration is highly desirable.

[00299] It is well established that lack of dystrophin expression is the primary genetic defect in DMD and BMD. However, the key mechanism leading to progressive muscle damage is largely unknown. It has been suggested that elevations of intracellular Ca^{2+}

concentrations ($[Ca^{2+}]_i$) under resting conditions directly contributed to toxic muscle cell (myofiber) damage and concurrent activation of Ca^{2+}-dependent proteases. Since calpain activity is increased in necrotic muscle fibers of mdx mice and calpain dysfunction contributes to limb-girdle muscular dystrophy, preventing activation of calcium-dependent proteases by inhibiting intracellular Ca^{2+} elevations represents a strategy to prevent muscle wasting in DMD. Significant differences in $[Ca^{2+}]_i$ between normal and dystrophic muscles have been reported in myotubes and animal models including the dystrophin-deficient mdx mouse. Intracellular Ca^{2+} elevations are prevented by administration of a pharmaceutical composition comprising a compound of the invention.

[00300] The compounds of the present invention are prepared in different forms, such as salts, hydrates, solvates, complexes, pro-drugs or salts of pro-drugs and the invention includes all variant forms of the compounds.

[00301] The present invention further provides a composition, comprising radio labeled compounds of the invention. Labeling of the compounds of the invention is accomplished using one of a variety of different radioactive labels known in the art. The radioactive label of the present invention is, for example, a radioisotope. The radioisotope is any isotope that emits detectable radiation including, without limitation, ^{35}S, ^{125}I, ^{3}H, or ^{14}C. Radioactivity emitted by the radioisotope can be detected by techniques well known in the art. For example, gamma emission from the radioisotope is detected using gamma imaging techniques, particularly scintigraphic imaging.

[00302] By way of non-limiting example, radio-labeled compounds of the invention are prepared as follows. A compound of the invention is demethylated at the phenyl ring using BBr_3. The resulting phenol compound then is re-methylated with a radio-labeled methylating agent (such as ^{3}H-dimethyl sulfate) in the presence of a base (such as NaH) to provide ^{3}H-labeled compounds.

[00303] Using forced swimming as an efficient protocol to increase skeletal muscle aerobic capacity in mice, the composition and phosphorylation status of the skeletal RyR1 channel complex have been investigated. Unexpectedly, after 3 weeks of 90 mins swimming twice daily, C57Bl6 wild-type mice showed significantly increased RyR1 phosphorylation by PKA while Ca^{2+}-calmodulin kinase II (CaMKII) phosphorylation was not changed indicating specificity of the stress pathway RyR1 protein expression was stable, however, RyR1

channels were depleted of the stabilizing subunit calstabin1 (FKBP12). It has been shown that RyR1 hyperphosphorylation and calstabin1 depletion are consistent with leaky RyR1 channels that cause intracellular SR Ca^{2+} leak.

[00304] RyR1 channels are PKA hyperphosphorylated and depleted of the stabilizing calstabin1 subunit after 3 weeks of 90 mins swimming twice daily. The immunoprecipitated RyR1 macromolecular channel complex shows increased PKA phosphorylation at Ser-2844 (corresponding to human RyR1-Ser-2843) whereas CaMKII phosphorylation at Ser-2849 (corresponding to human RyR1-Ser-2848) is unchanged. Concomitant with increased RyR1-Ser-2844 PKA hyperphosphorylation, calstabin1 is depleted from the channel complex. Normalization of phosphorylation and calstabin1 content to four subunits of the tetrameric channel complex shows a significant in increase in PKA phosphorylation and depletion of the stabilizing calstabin1 subunit.

Example 1: Effect of S36

[00305] The RyCal compounds referred to as S36, S107 were synthesized as described in co-pending applications USSN 11/212,309 and PCT/US2006/32405.

[00306] Figures 1-6 show some aspects of the molecular mechanisms which lead to muscle fatigue and the effect of S36 on muscle fatigue.

[00307] Drug Delivery: Eight-week-old, wild-type, weight-matched, C57BL/6J littermate mice were randomized to either S36 or vehicle treatment. On day –2, osmotic infusion pumps (Alzet Model 2004, 200µl total volume, 0.25 µl/hr delivery, Durect, Cupertino, CA) filled with either 200 ul of PBS or 200 µl of S36 (10 µg/ul diluted in PBS) were implanted subcutaneously on the dorsal surface of the mice by a horizontal incision just behind the neck. Mice were allowed to recover for three days prior to the initiation of exercise. Standard food and water were provided ad libitum.

[00308] Exercise Protocols: Beginning on day 1, mice were exercised for three weeks by swimming 5 days/week and by running on a treadmill an additional 1 day/week.

[00309] Swimming Model: The daily swimming protocol consisted of swimming sessions twice-daily separated by one-hour rest periods. After an initial conditioning regimen lasting 5 days during which the swimming sessions were increased in 10 minute increments from 40 minutes each to 80 minutes each, the swimming sessions thereafter lasted 90 minutes

each. A 30 cm wide by 30 cm long opaque acrylic tank was filled with tap water to a depth of at least 20 cm. Water was circulated and warmed to 32-34 degrees C using a separate reservoir with heating element, thermostat, and pump. Compressed room air was bubbled from Tygon tubing with small needle holes placed at the bottom of the tank to agitate the water surface. 4 mice, matched pair-wise with respect to treatment group, swam at any one time in the tank. Littermates who did not exercise were reserved as negative controls.

[00310] In order to track the swimming activity of each individually identified mouse, a video tracking system was used (San Diego Instruments, San Diego, CA), which includes Sony CCD video recorder, DVD/Hard Drive, frame grabber card, and custom SMART 2.0 software with Social Behavior package capable of tracking up to 8 mice simultaneously under ideal conditions.) Mice were anesthetized by using 1.5% isoflurane in O_2, and small, 0.75 cm, Velcro coins were sutured with 5-0 nylon suture to the scalp of each mouse. Plastic 1 cm colored dots, glued to the hook side of the Velcro, could be securely attached to the mouse and used for multiple subject tracking under appropriate lighting conditions. Each resultant mouse track in x,y, and time was analyzed and mean velocities, and distance swam over 2, 5, and 10 minute intervals was obtained.

[00311] Treadmill running: A Columbus Instruments(Columbus, OH) treadmill (Model: Exer-6M Treadmill with Treadmill Shock Detection Unit) with 6 lanes was used to run the mice. Mice were placed in their respective lanes at the lowest speed (7 meters/min) with the shocking apparatus turned off and allowed to adjust to the surroundings for 6 minutes. The forward half of the treadmill was covered with aluminum foil to block out light. A desk lamp illuminated the shocking area at the rear of the treadmill. After the adjustment period, the electric current was turned on, and the number of shocks delivered during the next two three minute intervals(training period) were recorded. The shock counter was then reset, the speed was increased to 10 m/min, and visits to the shocking area and shocks delivered to each mouse were recorded at three-minute intervals until the end of the experiment. At regular intervals, the speed of the treadmill was ramped up from the initial 10 m/min to as high as 36 m/min. The speed was increased no more than 2 m/min every 6 minutes. Consistent treadmill speed increases were used for all mice on a given day, but the protocol increased in difficulty over the course of the 21 day experiment. Task failure was defined when a mouse could not continue running to avoid the shocking area and gave up or

when the mouse had received 200 cumulative shocks. In nearly all cases, these two times were very close to identical.

[00312] Muscle Isolation: Following the 21st and 22nd day of exercise, mice were swum a final time on a staggered schedule. Following 90 minutes of swimming, each mouse was immediately sacrificed by carbon dioxide inhalation and cervical dislocation. Blood was removed by intracardiac aspiration, spun down, and plasma was eluted and frozen in liquid nitrogen. Both extensor digitorum longus (EDL) muscles were exposed, moistened with Tyrode's solution, and 4-0 silk sutures were tied to the proximal and distal tendons and the muscles were dissected free. The muscles were perfused with Tyrode's solution containing 2.0 mM $CaCl_2$, bubbled with 100% O_2, warmed to 35 C, and hung on isometric force transducers(F-30, Harvard Apparatus, Cambridge, MA). After equilibration for 10 minutes at a resting tension of 1 cN and a brief potentiation protocol, force-frequency relationships were measured, with 60 second delays between 800 ms stimulations at 40-150 Hz. Fatigue was produced with a protocol of 50 Hz tetani (each 600 ms long) every 2 seconds for 120 seconds. DMC v4.1.6 (Aurora Scientific, Canada) was used to stimulate and record muscle responses, and DMA v3.2(Aurora Scientific, Canada) was used to analyze the resultant data.

[00313] Following stimulation, muscle length was determined at the resting tension, and muscle dry weight was recorded. One EDL muscle was frozen in isopentane (-80 C) for histology and the other was frozen in liquid N_2 for biochemistry.

[00314] In addition, both soleus muscles were dissected and likewise one was frozen in isopentane for histology and one was frozen in liquid N_2 for biochemical analysis. The vastus lateralis, heart, and diaphragm were also dissected from each animal and frozen in liquid N_2 for biochemical analysis.

[00315] Biochemistry: RyR channels were immunoprecipitated from skeletal muscle homogenates with anti-RyR antibody in 0.5 ml of buffer (50 mM Tris HCl buffer, pH 7.4 0.9% NaCl 5.0 mM NaF 1.0 mM Na3VO4 0.5% Triton X-100 + protease inhibitors) for 1 hour at 4°C. The samples were incubated with protein A Sepharose beads (Amersham Pharmacia) at 4°C for 1 h, after which the beads were washed three times with buffer. Proteins were separated on SDS PAGE gels (6% for RyR2 and 15% for calstabin2) and transferred onto nitrocellulose membranes overnight (SemiDry transfer blot, Bio-Rad). After incubation with 5% nonfat milk to prevent non-specific antibody binding and a wash in Tris-

buffered saline with 0.1% Tween-20, membranes were incubated for 1–2 h at room temperature with primary antibodies anti-calstabin(1:1,000), anti-RyR (5029; 1:5,000), or anti-phospho-RyR2-pSer2809 (1:5,000), which detects PKA-phosphorylated mouse RyR1-pSer2844 and RyR2-pSer2808. After three washes, membranes were incubated with horseradish peroxidase-labeled anti-rabbit IgG (1:5,000, Transduction Laboratories, Lexington, KY), and developed with an enhanced chemiluminescent detection system (Amersham Pharmacia). Band densities were quantified by using QUANTITY ONE software (Bio-Rad).

Example 2: Effect of S107

[00316] Figures 7-20 show molecular mechanisms of muscle fatigue and the effect of S107.

[00317] Drug Delivery: Eight-week-old, weight-matched, C57BL/6J littermate mice were randomized to dosing with either S107 or vehicle (H2O). On day -3 of each trial, osmotic pumps (Alzet Model 2004, 200ul total volume, 0.25 ul/hr delivery, Durect, Cupertino, CA) filled with either 200 ul of PBS or 200 ul of S107 (10 ug/ul diluted in H2O) were implanted subcutaneously on the dorsal surface of each mouse by a horizontal incision on the neck. Mice were allowed to recover for three days prior to the initiation of exercise. Standard food and water were provided ad libitum through the experiment.

[00318] Chronic Exercise Model: The daily swimming protocol consisted of twice-daily swimming sessions separated by a one-hour rest period. After an initial conditioning regimen lasting 5 days during which the swimming sessions were increased in 10 minute increments from 40 minutes each to 80 minutes each, the swimming sessions thereafter lasted 90 minutes each. A 30 cm wide by 30 cm long opaque acrylic tank was filled with tap water to a depth of at least 20 cm. Water was circulated and warmed to 32-34 degrees C using a separate reservoir with heating element, thermostat, and pump. 8 mice, balanced pair-wise with respect to genotype and/or treatment group, swam at any one time in the tank. Littermates who did not exercise were reserved as sedentary controls.

[00319] In order to confirm that uniform exercise conditions were achieved, pilot experiments were performed in which the motion of each individually identified mouse was tracked with a video tracking system(San Diego Instruments, San Diego, CA). Individual recorded tracks over the full 90 minutes of each swim were analyzed for distance swam, mean

velocities over time, etc. using the SMART 2.0 software with Social Behavior package (San Diego Instruments). No significant differences in the degree of exercise were noted.

[00320] Treadmill performance: A Columbus Instruments (Columbus, OH) treadmill (Model: Exer-6M Treadmill with Treadmill Shock Detection Unit) with 6 lanes was used to run the mice. Mice were placed in their respective lanes with the shocking apparatus turned off and allowed to adjust to the surroundings for 10 minutes. The forward half of the treadmill was covered with aluminum foil to block out light and a desk lamp illuminated the shocking area at the rear of the treadmill. After the adjustment period, the treadmill was set to 10 meters/min, and the mice were trained to run with gentle prodding for 6 minutes. The electric current was then turned on, and the number of shocks delivered during the next two three minute intervals (training period) were recorded. The shock counter was then reset and visits to the shocking area and shocks delivered to each mouse were recorded at three-minute intervals until the end of the experiment. At regular intervals, the speed of the treadmill was ramped up from the initial 10 m/min to 24 m/min. The speed was increased no more than 2 m/min every 6 minutes. Task failure was defined when a mouse could not continue running despite gentle prodding.

[00321] Intact muscle preparation: Immediately following a forced exercise session, each mouse was sacrificed by carbon dioxide inhalation and cervical dislocation. Blood was removed by intracardiac aspiration, spun down, and plasma was eluted and frozen in liquid nitrogen. 4-0 silk sutures were tied to the proximal and distal tendons of intact EDL and soleus muscles and the muscles were dissected free and placed in a modified Ringer's solution(140 mM NaCl, 5 mM Kcl, 2.0 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.4) bubbled with 100% O_2. Muscles were hung vertically in 50 mL Radnoti jacketed glass chambers with one tendon attached with 4-0 silk suture to an isometric force transducer (F30, Harvard Apparatus, Cambridge, MA) and the other tendon attached by suture to a stationary arm with built in platinum stimulating plate electrodes. After perfusion with 35C Ringer's and equilibration for 10 minutes at a resting tension of 1 cN and a brief potentiation protocol, force-frequency relationships were measured, with 60 second delays between 800 ms stimulations at 40-150 Hz. DMCv4.1.6 (Aurora Scientific, Canada) was used to stimulate and record muscle responses, and DMA v3.2(Aurora Scientific, Canada) was used to analyze the resultant data. Following stimulation, muscle length was determined at resting tension, and muscle dry weight was recorded.

[00322] Confocal microscopy: Single flexor digitorum brevis (FDB) fibers were enzymatically dissociated by standard methods (Reiken, Lacampagne et al. 2003). Briefly, the muscle was dissected from the paw, placed in a modified Ringer's solution, and stripped of all fascia. Type 1 collagenase (2 mg/ml, Sigma) in Ringer's solution was prepared fresh, and the muscle was digested for 2 hours at 37C in a incubator shaking at 125 rpm. The muscle was placed in fresh Ringer's and gently triturated. Single fibers were collected and allowed to attach to glass coverslips coated in laminin (Sigma L-2020). The cells were loaded with 2μM fluo4-AM ester (Invitrogen) for 20 minutes, placed on the Zeiss Live 5 microscope stage and superfused for 15 minutes with Ringer's. The fibers were paced at 1 Hz for 10 minutes prior to imaging baseline fiber properties. Linescan images were continuously acquired at 1 ms scan rate during a tetanic sequence consisting of 300 ms stimulation at 100 Hz with a 2 Hz train rate. Images were analyzed in ImageJ, and an F/F0 ratio was calculated for each fiber.

[00323] Human Exercise Protocol: Three weeks prior to test sessions, subjects reported to the Human Performance Laboratory at Appalachian State Univeristy for baseline measurements of cardiorespiratory fitness and body composition. On three consecutive test session days, subjects ate a standardized breakfast (7 - 8:00 am) and lunch (completed by 12:30 pm), and then reported to the ASU Human Performance Laboratory at 2:00 pm. Subjects exercised on exercise bikes at 70% VO_{2max} from 3:00-6:00 pm. Test sessions days were Monday, Tuesday, and Wednesday afternoons. Oxygen consumption and other metabolic parameters were measured using a metabolic cart (with a mouthpiece and noseclip) every 30 minutes, and blood lactate and glucose (via finger stick) every 60 minutes to verify that subjects were adhering to the prescribed exercise workloads. Subjects ingested 0.5-1.0 liters water every hour of exercise while avoiding all forms of ingested energy (e.g., bars, drinks). Resting control subjects sat in the laboratory during the exercise test sessions. Blood, urine, and saliva samples were collected 15-30 minutes before exercise/sitting, and then within 5-10 minutes post-exercise on each of the 3 test sessions. Muscle biopsy samples were obtained 15-30 minutes before exercise/sitting, and then within 5-10 minutes post-exercise using a needle biopsy procedure on Days 1 and 3. Four samples were taken (two from each thigh), about 2 inches apart. Biopsies were snap frozen in liquid nitrogen and stored at -80C.

[00324] Single channel recording and data acquisition: SR vesicles from skeletal muscle of sedentary mice and mice chronically exercised and treated either with vehicle or S107 were prepared as described previously (Reiken, Lacampagne et al. 2003). RyR1 channels were reconstituted by spontaneous fusion of microsomes into the planar lipid bilayer (a mixture of phosphatidylethanolamine and phosphatidylserine in a 3:1 ratio, Avanti Polar Lipids). Planar lipid bilayers were formed across a 200 μm aperture in a polysulfonate cup (Warner Instruments, Inc.), which separated two bathing solutions (1 mM EGTA, 250/125 mM HEPES/Tris, 50 mM KCl, 0.5 mM $CaCl_2$, pH 7.35 as *cis* solution and 53 mM $Ba(OH)_2$, 50 mM KCl, 250 mM HEPES, pH 7.35 as *trans* solution). After incorporation, RyR1 channel activity was recorded continuously for at least 10 minutes. The concentration of free Ca^{2+} in the *cis* chamber was calculated with WinMaxC program (version 2.50) (Bers, Patton et al. 1994). Single channel currents were recorded at 0 mV using the Axopatch 200A patch-clamp amplifier (Axon Instruments, USA) in gap-free mode, filtered at 1 kHz, and digitized at 10 kHz. Data acquisition was performed using Digidata 1322A and Axoscope 9 software (Axon Instruments, USA). The recordings were analyzed using Clampfit 10.1 (Molecular Devices, USA) and Origin software (ver. 6.0, Microcal Software, Inc., USA).

[00325] Analysis of Ryanodine Receptor Complex: 10 mg muscle samples were isotonically lysed. The ryanodine receptor (RyR1) was immunoprecipitated by incubating 250 μg of homogenate with anti-RyR antibody (2 μl 5029 Ab) in 0.5 ml of a modified RIPA buffer (50 mM Tris-HCl pH 7.4, 0.9% NaCl, 5.0 mM NaF, 1.0 mM Na_3VO_4, 0.5% Triton-X100, and protease inhibitors) for 1 hr at 4°C. The samples were incubated with protein A Sepharose beads (Amersham Pharmacia) at 4°C for 1 h, after which the beads were washed three times with buffer. Proteins were separated on SDS-PAGE gels (4-20% gradient) and transferred onto nitrocellulose membranes overnight (SemiDry transfer blot, Bio-Rad). After incubation with blocking solution (LICOR Biosciences, Lincoln NE) to prevent non-specific antibody binding and a wash in Tris-buffered saline with 0.1% Tween-20, membranes were incubated for 1-2 h at room temperature with primary antibodies anti-calstabin (1:2500 in blocking buffer), anti-RyR (5029, 1:5,000), or anti-phospho-RyR2-pSer2809 (1:5000), which detects PKA-phosphorylated mouse RyR1-pSer2844 and RyR2-pSer2808, anti-PDE4D3 (1:1000). After three washes, membranes were incubated with infrared labeled secondary antibodies (1:10,000 dilution, LICOR Biosystems). Band densities were quantified using the Odyssey Infrared Imaging System (LICOR Biosciences).

[00326] Calpain and Creatine Kinase Assays: Tissue calpain activities were measured using a calpain activity assay kit (Calbiochem, San Diego, CA). This assay is based on the degradation of the fluorescent peptide substrate Suc-LLVY-AMC (Calbiochem). Muscle homogenates were diluted to a final concentration of 600 μg/ml and the calpain activity in the homogenate was determined as per manufactures instructions. Plasma creatine kinase (CK) activity was assayed using the regent kit from Pointe Scientific, Inc. (Canton, MI). Plasma samples (duplicates, 5 μl each) were added to 200 μl of CK reagent and the change in absorbance at 340 nM was recorded over 4 minutes using a plate reader. The average absorbance change per minute was used to determine the CPK levels as per manufacturer's instructions.

[00327] Statistics: Data are presented as mean ± SEM. Independent t-test with a significance level of 0.05 was employed to test differences between cal1-/- and WT, PDE4D-/- and WT, and Ex + veh and Ex + S107, except as noted below. The distributions of treadmill failure data were found in several cases to be asymmetric. As such, Wilcoxon rank sum tests were used for all such data comparisons.

[00328] High intensity exercise induces RyR1 PKA hyperphosphorylation, and depletion of calstabin1 and PDE4D3 from the channel complex: Exercise models in the mouse have been grouped into two categories: 1) voluntary exercise including running wheels; and 2) involuntary exercise, including swimming or forced treadmill runs to exhaustion. In order to achieve high intensity exercise, a twice daily swimming protocol was adapted to achieve uniform exercise in mice over days to weeks. This mouse exercise protocol was not designed to be either explicitly eccentric or isometric in character, but rather a physiologic mix of eccentric and isometric exercise. Following forced exercise, RyR1 was immunoprecipitated out of whole muscle homogenates from hind limb muscles, and the RyR1 channel complex was size-fractionated on SDS-PAGE and immunoblotted for channel complex components. High-intensity exercise in the mouse resulted in progressive phosphorylation of RyR1 at the PKA site Ser2844 (RyR1-pS2844) that saturated by 14 days of twice daily swimming (90 min sessions, e.g. Fig. 8A).

[00329] In addition, the RyR1 macromolecular complex from extensor digitorum longus (EDL) muscle underwent remodeling, including depletion of calstabin1 and PDE4D3 from the channel by day 14 (Fig. 8A and 8B). An identical pattern of biochemical changes

was seen in all other hind limb skeletal muscles isolated from the same mice, including soleus, tibialis anterior, and gastrocnemius. RyR1 PKA hyperphosphorylation and depletion of calstabin1 and PDE4D3 were dependent on the intensity of the exercise, with only relatively high intensity exercise resulting in significant channel modifications (Fig 8C and 8D). Furthermore, the remodeling of the RyR1 channel complex persisted after exercise and recovered only partially following three days of rest after chronic exercise (Fig. 16A). PKA hyperphosphorylation of RyR1 was not due to changes in the amount of PKA and PP1 bound the RyR1 complex, as these levels were not affected by exercise conditions (Fig. 16A and 16B). Calstabin1 levels in whole muscle homogenate, measured by immunoblot, were not altered during exercise (Fig. 16C). Thus, intense daily exercise, over weeks causes remodeling of the RyR1 channel complex manifested by depletion of the phosphodiesterase PDE4D3 from the channel, PKA hyperphosphorylation, and depletion of the stabilizing subunit calstabin1 from the RyR1 channel complex.

[00330] RyR1 channel defects occur during human exercise: To assess whether the remodeling of the RyR1 channel macromolecular complex observed in exercised mice is relevant to human physiology, human thigh muscle biopsies were obtained from trained athletes before and after exercise on Days 1 and 3 of a high-intensity exercise protocol (cycling 3 hr/day at 57% of VO_2max)(Nieman, Henson et al. 2006). The human RyR1 macromolecular complex was immunoprecipitated from muscle homogenate, size fractionated by SDS-PAGE, and immunoblotted to assess RyR1 PKA phosphorylation and levels of calstabin1 and PDE4D3 in the RyR1 complex. High intensity exercise resulted in PKA hyperphosphorylation of RyR1 and calstabin1 depletion compared to controls who did not exercise (Fig. 9). Prior to exercise on Day 3, PKA phosphorylation of RyR1 in the trained cyclists was at or near resting levels and no significant calstabin1 depletion from the RyR1 complex was observed, however, PDE4D3 was stably depleted from the RyR1 complex by the beginning of the third day of the high intensity exercise (Fig 9B). Thus, the same remodeling of the RyR1 channel complex observed in chronically exercised mice occurs in highly trained athletes subjected to intense exercise.

[00331] Muscle-specific calstabin1-/- mice have a high-intensity exercise defect: Muscle-specific deficiency of calstabin1 has been previously shown to be associated with alterations in the force-frequency relationships of isolated muscle preparations and a reduction in Cav1.1 current (Tang, Ingalls et al. 2004). To determine if calstabin1 binding to RyR1 has

an effect on exercise performance, we assessed treadmill run to exhaustion times in mice with muscle-specific deficiency of calstabin1 (cal1-/-). There was a significant defect in the high intensity exercise capacity of cal1-/- mice compared to WT littermate controls (Fig. 10A). The exercise defect was similar in both males and females (Fig. 10B), and despite a small reduction in the body weights of the cal1-/- mice (Fig 10C), there was no correlation between failure time and reduced body weight (Fig. 10D). The exercise defect was most apparent in high intensity exercise (treadmill speeds equal to or greater than 24 m/min) or in eccentric exercise such as 14 degree downhill treadmill runs. 0/2 cal1-/- mice were able to complete a 30 min downhill treadmill exercise protocol while 2/2 WT littermates were able to complete the protocol.

[00332] Twenty-four hours following a downhill exercise regimen (as described herein), plasma creatine kinase (CPK), was elevated consistent with increased muscle damage in the *cal1-/-* mice compared to WT littermate controls (Fig 10E). Following several weeks of daily exercise training, the exercise capacity of WT mice approached that of *cal1 -/-* presumably because of the progressive depletion of calstabin1 from the RyR1 complex that occurs with chronic exercise in WT mice (Fig. 8) resulting in an exercised-induced depletion of calstabin1 from the RyR1 channel complex that is comparable to that observed prior to exercise in the *cal1-/-* mice (Fig 10F). Thus, muscle-specific calstabin1 deficient mice exhibit enhanced fatigue consistent with depletion of calstabin1 from the RyR1 complex playing a role in muscle fatigue.

[00333] PDE4D -/- mice exhibit an exercise defect: Global deficiency of PDE4D results in an age-dependent progressive cardiomyopathy (Lehnart, Wehrens et al. 2005). Prior to the age of three months, however, PDE4D-/-mice exhibit no cardiac defect as determined by echocardiogram or cardiac catheterization. The exercise capacity of 2 month-old PDE4D-/- mice was compared with their WT littermates. A significant reduction in exercise capacity was observed (Fig. 11A) without any correlation with body weight (Fig. 11B,C,D). Remarkably, CPK levels were increased, consistent with muscle damage at rest, in PDE4D-/ mice relative to WT littermate controls, and there was a significant increase in CPK levels 24 hours following a single episode of eccentric exercise consisting of thirty minutes of downhill treadmill running (Fig. 11E) in PDE4D-/- mice. PDE4D-/- mice exhibited an absence of RyR1 bound PDE4D3, a small increase in basal RyR1 PKA phosphorylation, and a significant increase in calstabin1 depletion following only a single day

of mild exercise (Fig. 11F). These data show that PDE4D3 plays a significant role in the RyR1 complex by regulating the extent of PKA phosphorylation of RyR1 and that depletion of PDE4D3 from the channel complex during exercise promotes PKA phosphorylation of RyR1 which in turn contributes to muscle damage and skeletal muscle fatigue during exercise.

[00334] Pharmacologic rebinding of calstabin1 to RyR1 improves chronic exercise performance: Having demonstrated that a deficiency of calstabin1 is associated with an exercise defect and that chronic or high intensity exercise can result in calstabin1 depletion from RyR1, the effect on chronic or high intensity exercise performance of pharmacologic rebinding of calstabin1 to RyR1 was determined. 1,4-benzothiazepine derivatives, RyCal compounds, were screened to identify compounds with increased target activity, improved specificity (absence of activity against other known ion channels) and *in vivo* efficacy in terms of improved exercise capacity. Compound S107 at a concentration of 500 nM was found not to affect L-type calcium channel current or hERG potassium current .

[00335] Age and sex-matched WT mice were randomized to receive osmotic pumps containing either S107 or vehicle. Dosing with S107 at 2.5 ug/hr or vehicle was initiated four days prior to the beginning of a 21-day forced swimming exercise protocol. Exercise capacity was assessed once a week by a level treadmill run to exhaustion during the nocturnal cycle of the mouse. The mice were not exercised on the same day as treadmill assessments. Fig. 12A shows that calstabin1 rebinding with S107 had no acute effect on WT exercise performance, but that over time the S107 treated WT mice were relatively protected against a decline in treadmill exercise capacity that occurred in vehicle treated mice ($p<0.05$ Wilcoxon rank test, S107 vs vehicle at Day 21). These studies are complicated by the training effect of repeated exercise which leads to improved performance due to enhanced musculature. Individual treadmill failure times on Day 21 are shown in Fig. 12B. Isometric force production was measured in EDL muscles in a tissue bath during field stimulation.

[00336] EDL muscles from S107 treated mice showed increased force production at stimulation frequencies greater than 80 Hz consistent with a left-shift of the force-frequency relationship (Fig. 12C). Drug treatment did not result in a change in body weight (Fig. 12D) or muscle weight. In parallel chronic exercise trials with mice deficient in calstabin1, S107 failed to improve treadmill performance (Fig. 12E). The chronic exercise protocol resulted in

substantial PKA phosphorylation of RyR1 and calstabin1 depletion from immunoprecipitated RyR1. Calstabin1 depletion from RyR1 was nearly entirely reversed by S107 treatment (Fig. 12F). Taken together these data show that preventing the SR Ca^{2+} leak due to PKA hyperphosphorylated RyR1 channel with a drug that enhances calstabin1 binding to the channel can protect against muscle damage, muscle fatigue, enhance muscle function and improve exercise performance during fatigue protocols.

[00337] Reduced fatiguability in calstabin1 rebound FDB muscle fibers: Flexor digitorum brevis (FDB) muscle fibers were enzymatically dissociated from mice following the chronic exercise protocol and loaded with the calcium indicator fluo-4. Individual muscle fibers were imaged on a Zeiss Live5 confocal microscope during field stimulation at 1 Hz and during a fatiguing protocol consisting of repeated 300 ms long 120 Hz tetani every 2 seconds for 400 seconds. Representative F/F0 traces during the fatigue protocol are shown for a FDB fiber isolated from a vehicle treated mouse (Fig. 13A) and a S107 treated mouse (Fig. 13B). FDB fibers from S107 treated mice exhibited a delayed decline in peak tetanic calcium transients (Fig. 13C). It is known that muscle fibers with slower kinetics of Ca^{2+} release and reuptake are less prone to fatigue. The kinetics of Ca^{2+} release and reuptake during single twitches at 1 Hz were assessed. The distribution of 50% reuptake times (tau) showed no significant differences between vehicle and S107 treatment (Fig. 17), indicating no shift in the calcium reuptake kinetics of the FDB fibers. These data indicate that treatment with S107 improves Ca^{2+} handling in muscle fibers during fatigue protocols.

[00338] Chronic exercise results in leaky RyR1 Channels which can be reversed by calstabin1 rebinding: A critical issue is whether the biochemical changes in the RyR1 macromolecular complex identified during exercise result in changes in RyR1 channel activity. To address this directly, SR microsomes were prepared from the hind limb muscle of sedentary mice, mice chronically exercised and treated with vehicle, and mice chronically exercised and treated with S107. Using standard techniques, vesicles were fused to planar lipid bilayers and the single channel activity of incorporated RyR1 channels was continuously measured for at least 10 minutes at 90 nM $[Ca^{2+}]_{cis}$ (Fig. 14A). In agreement with previously published data (Meissner, 1994, Reiken, 2003), the activity of RyR1 from sedentary mice at resting calcium concentrations was very low resulting in a small number of openings over long period of time (in some experiments as long as 20 min of recording was necessary to calculate an open probability for the channel). In contrast, RyR1 channels

from mice chronically exercised and treated with vehicle displayed increased activity with significantly higher open probabilities ($p < 0.005$, Ex + veh, $n = 9$ vs sedentary, $n = 9$) due to an increased frequency of openings (Fig. 14B). Administration of S107 caused a significant decrease of RyR1 open probability ($p < 0.005$, Ex + S107, $n = 12$ vs Ex + veh, $n = 9$) to a level comparable to that observed in channels from sedentary mice. Neither chronic exercise nor S107 had any effect on the duration of the channel dwell times meaning that observed changes in open probability were due to changes in the number of opening events (Fig 14B). These data show that RyR1 channels from exercised animals exhibit "leaky" channel behavior (increased open probability) and that channels from animals treated with S107 were not "leaky".

[00339] Reduced calpain activation in muscle tissue and reduced muscle tissue damage due to calstabin1 rebinding: One possible mechanism by which calcium released by leaky RyR1 channels impairs exercise performance is the activation of a member of the calpain family of Ca^{2+}-dependent neutral proteases, which are known to be responsible for muscle damage in a number of pathophysiological states. (Belcastro 1993; Berchtold, Brinkmeier et al. 2000). Calpain activity in muscle homogenates was assessed by means of the degradation of the synthetic calpain substrate Suc-LLVY-AMC, which fluoresces upon cleavage by calpain. Chronically exercised EDL muscle exhibited elevated calpain activity compared to sedentary controls. Calpain activity was significantly reduced in S107 treated and chronically exercised mice (Fig. 15A). Evidence of a protection from muscle damage was further provided by measurement of plasma CPK activity levels which were elevated in the chronically exercised mice, but reduced close to the levels observed in sedentary controls in the S107 treated mice (Fig. 15B). Muscle histology showed evidence of muscle hypertrophy with some inflammation and scattered loci of damaged fibers in the chronically exercise mice, without evidence of extensive necrosis in either treatment group.

Example 3: Muscular dystrophy and effects of S107

[00340] RyR1 calcium release channels become PKA hyperphosphorylated and depleted of the stabilizing protein calstabin1 during exercise. The compounds of the invention increase the binding affinity of calstabin1 to PKA hyperphosphorylated RyR1. These compounds (referred to as called "calcium channel stabilizers" or "rycal") are 1,4-benzothiazepines and derivatives thereof. Treatment with these compounds improves exercise performance of mice running on a treadmill. A calcium leak via PKA

hyperphoshorylated RyR1 channels causes muscle damage due to activation of calcium-dependent proteases and rycals prevent the calcium leak and inhibit muscle damage during chronic exercise. Rycals can be used to improve muscle fatigue in chronic diseases including heart failure, AIDS, cancer, renal failure, and can also be used to treat muscular dystrophies.

[00341] Duchenne muscular dystrophy (DMD) is an X-linked muscle disease characterized by mutations in the dystrophin gene. Increased calcium-activated calpain proteolysis in the sarcolemma membrane is thought to be a primary mechanism in the pathophysiology of DMD. The *mdx* mouse, carrying a stop codon inside exon 23 of the dystrophin gene, provides a useful system to study the effectiveness of different therapeutic strategies for the cure of this disease.

[00342] A RyCal compound, S107, reduces calpain activity in mdx mice during exercise, Figures 7, 18, 19, 20. This indicates that RyCals may be useful for treating muscle related diseases, including, but not limited to, muscular dystrophies.

[00343] Animal Model: *mdx* mice (21 days old) were treated with S107 (0.125 mg/kg/h) or vehicle using implantable, osmotic pumps for 28 days. After treatment, the mice were subjected to downhill (14degree) treadmill running for 30 min at 18 m/min. Immediately after the exercise, the animals were sacrificed and the skeletal muscles were harvested.

[00344] Preparation of EDL Homogenates: EDL muscles homogenates were prepared in 0.5 ml of homogenization buffer (20 mM NaF, 10 mM Tris-maleate, pH7.2 + protease inhibitors). Cardiac sarcoplasmic reticulum (CSR) fractions were obtained by centrifuging the homogenates at 50,000 x g for 30 min. Homogenates were centrifuged at 4000 x g for 20 min and the supernatants were centrifuged for 20 min at 10000 x g. Aliquots of the homogenates were assayed for protein concentration and stored at -80°C.

[00345] Calpain Activity Assay: Calpain activity of EDL homogenates (30 μg) were measured using a Calpain Activity Assay kit (Calbiochem). The assay utilizes a synthetic calpain substrate, suc-LLVY-AMC. AMC is released upon cleavage with calpain and is measured fluorometrically. Assays are performed with both an activation and an inhibition buffer to determine specific calpain activity in the sample.

[00346] Serum Creatine Kinase: Serum (10 µl) proteins were separated using 4-20% PAGE. After transferring the proteins to nitrocellulose, the immunoblots were developed using an anti-creatine kinase Antibody (Research Diagnostics, 1:1000 dilution). Bands were quantified by densitometry.

[00347] Immunoprecipitation of Ryanodine Receptor: The ryanodine receptor (RyR1) was immunoprecipitated from samples by incubating 250 µg of EDL homogenate with anti-RyR antibody (2 µl 5029 Ab) in 0.5 ml of as modified RIPA buffer (50 mM Tris-HC1 (pH 7.4), 0.9% NaCl, 5.0 mM NaF, 1.0 mM $Na_3 VO_4$, 0.5% Triton-X100, and protease inhibitors) 1 hr at 4°C. The samples were incubated with Protein A sepharose beads (Ammersham Pharmacia Biotech, Piscatawy, NJ) at 4°C for 1 hour, after which, the beads were washed three times with RIPA. Samples were heated to 95C and size fractionated by PAGE.

[00348] Western Analysis: Samples (immunoprecipitates or 10 µg CSR) were heated to 95° C and the proteins were size fractionated on 6% SDS PAGE for RYR and 15% PAGE for calstabin. Immunoblots were developed using antibodies against RyR (5029, 1:5000 dilution), PKA phosphorylated RyR (1:10000), or Calstabin (1:2000). Dilutions are made in 5% milk in TBS-T.

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Zhang, S.-J., J. D. Bruton, et al. (2006). "Limited oxygen diffusion accelerates fatigue development in mouse skeletal muscle." J Physiol (Lond) 572(2): 551-559.

What is claimed is:

1. A method for treating or preventing muscle fatigue in a subject, the method comprising administering to the subject a therapeutically effective amount of a compound represented by the structure of Formula I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof,

wherein the compound is not S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, or JTV-519.

2. The method of claim 1, wherein the compound is represented by the structure of Formula I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof.

3. The method of claim 1, wherein the compound selected from the group consisting of S101, S102, S103, S104, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123 any salt, hydrate, solvate, polymorph, complex, metabolite, pro-drugs thereof, and any combination thereof.

4. The method of any one of claims 1-3, wherein the muscle fatigue is due to a skeletal muscle disease or disorder.

5. The method of claim 4, wherein the skeletal muscle disease or disorder is associated with abnormal function of an Ryanodine 1 receptor (RyR1).

6. The method of claim 4, wherein the skeletal muscle disease or disorder is a myopathy.

7. The method of claim 6, wherein the myopathy is a muscular dystrophy, central core disease, or malignant hyperthermia.

8. The method of any one of claims 1-3, wherein the muscle fatigue is due to a disease or condition selected from the group consisting of: neuropathy, a neurological disease or

disorder, a seizure condition, a genetic disease or disorder, a cardiac disease or disorder, an infectious disease, HIV infection, AIDS, cancer, malnutrition, renal disease, and renal failure.

9. The method of claim 8, wherein the cardiac disease or disorder is selected from the group consisting of an irregular heartbeat condition, an exercise-induced irregular heartbeat, congestive heart failure, chronic obstructive pulmonary disease, high blood pressure or any combination thereof.

10. The method of claim 9, wherein the irregular heartbeat condition is selected from the group consisting of atrial or ventricular arrhythmia, atrial or ventricular fibrillation; atrial or ventricular tachyarrhythmia; atrial or ventricular tachycardia, catecholaminergic polymorphic ventricular tachycardia (CPTV), and exercise-induced variants thereof.

11. The method of any one of claims 1-3, wherein the muscle fatigue is exercise-induced muscle fatigue.

12. The method of claim 11, wherein the exercise-induced muscle fatigue is due to prolonged exercise or high-intensity exercise.

13. The method of claim 1, wherein the subject is a non-human animal selected from the group consisting of a canine, an equine, a feline, a porcine, a murine, a bovine, an avian and an ovine animal.

14. The method of claim 1, wherein the subject is a human and the compound is included in a pharmaceutical composition that comprises at least one pharmaceutically acceptable excipient.

15. The method of claim 14, wherein the at least one pharmaceutically acceptable excipient is selected from the group consisting of aromatics, buffers, binders, colorants, disintegrants, diluents, emulsifiers, extenders, flavor-improving agents, gellants, glidants, preservatives, skin-penetration enhancers, solubilizers, stabilizers, suspending agents, sweeteners, tonicity agents, vehicles, and viscosity-increasing agents, or any combination thereof.

16. The method of claim 15, wherein the pharmaceutical composition further comprises a second active agent.

17. The method of claim 16, wherein the second active agent is an analgesic.

18. The method of claim 17, wherein the composition is in a capsule form, a granule form, a powder form, a solution form, a suspension form, or a tablet form.

19. The method of claim 18, wherein the composition is administered orally, parenterally, enterally, intravenously, intraarterially, subcutaneously, intramuscularly, via a drug releasing stent or an implant, or via an osmotic pump.

20. The method of claim 1, wherein the compound is administered to the subject at a dose sufficient to reduce calpain activity.

21. The method of claim 1, wherein the compound is administered to the subject as a dose sufficient to reduce plasma creatine kinase activity or levels.

22. Use of a therapeutically effective amount of a compound represented by the structure of Formula I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof for preparing a composition for treating muscle fatigue in a subject,

wherein the compound is not S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, or JTV-519.

23. Use of a therapeutically effective amount of a compound represented by the structure of Formula I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites or pro-drugs thereof, or any combination thereof for preparing a composition for treating muscle fatigue in a subject.

24. The use of claim 22 or 23, wherein the compound selected from the group consisting of S101, S102, S103, S104, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123 any salt, hydrate, solvate, polymorph, complex, metabolite, pro-drugs thereof, and any combination thereof.

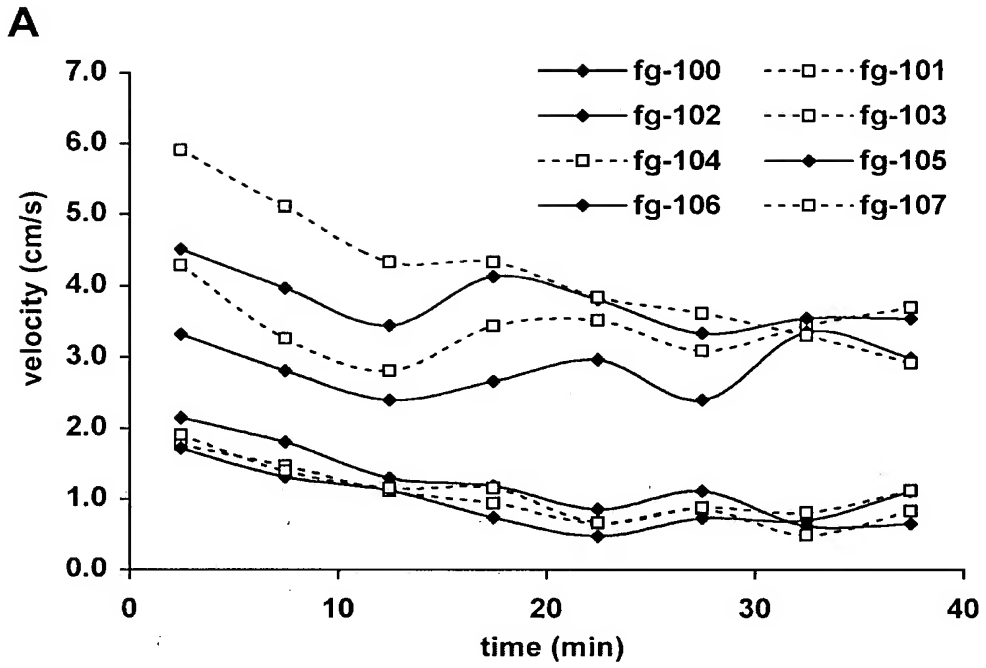

A
fg-100
fg-101
fg-102
fg-103
fg-104
fg-105
fg-106
fg-107
velocity (cm/s)
time (min)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
0
10
20
30
40

FIG. 1A

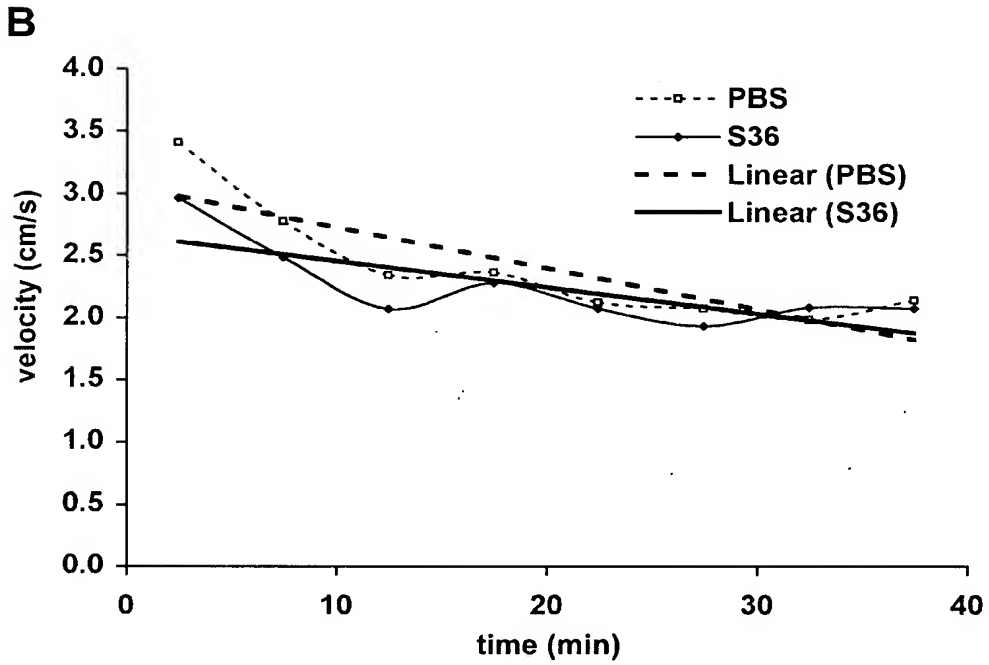

B
PBS
S36
Linear (PBS)
Linear (S36)
velocity (cm/s)
time (min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0
10
20
30
40

FIG. 1B

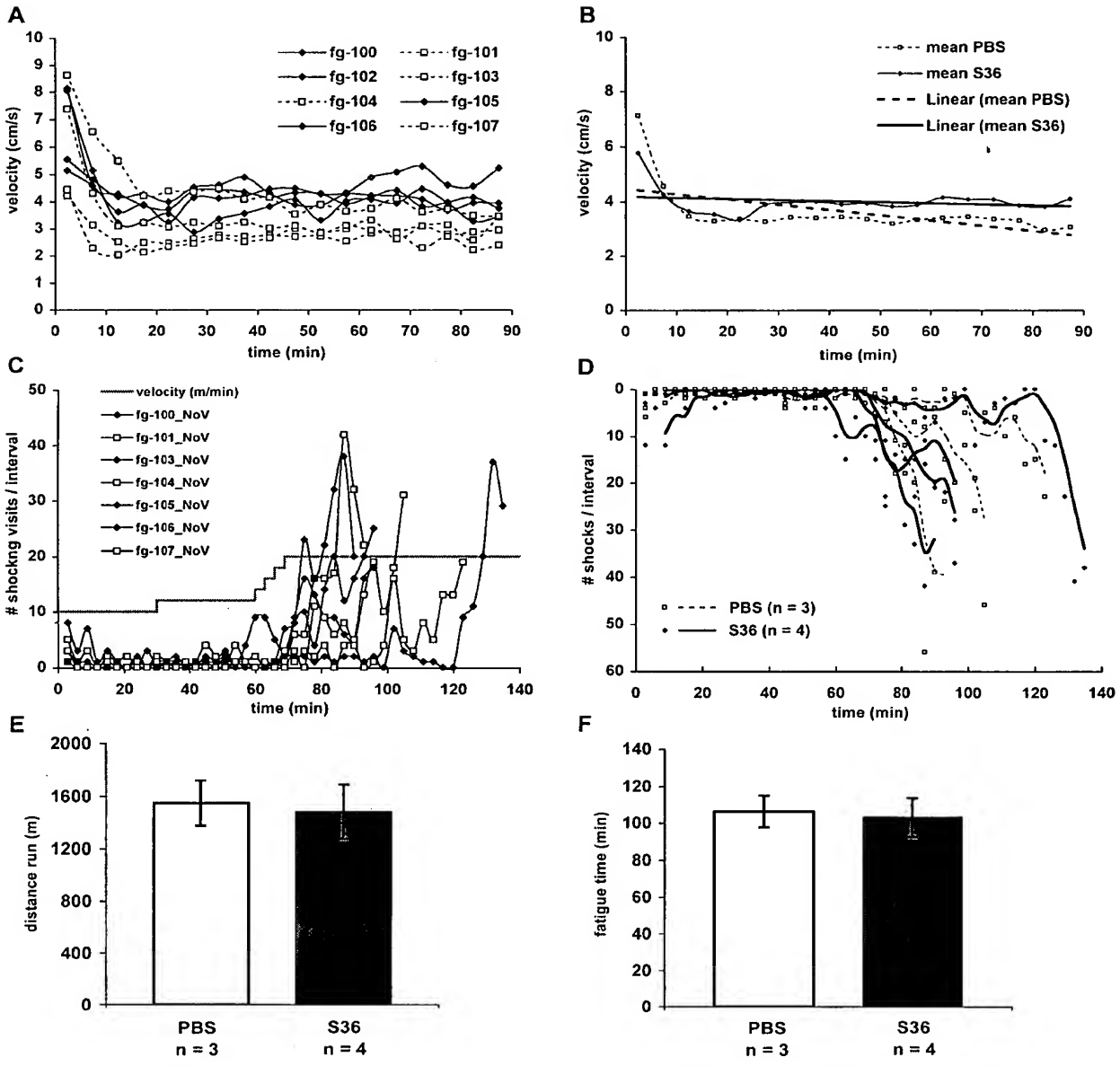

A
velocity (cm/s)
time (min)
fg-100
fg-101
fg-102
fg-103
fg-104
fg-105
fg-106
fg-107
B
velocity (cm/s)
time (min)
mean PBS
mean S36
Linear (mean PBS)
Linear (mean S36)
C
shockng visits / interval
time (min)
velocity (m/min)
fg-100_NoV
fg-101_NoV
fg-103_NoV
fg-104_NoV
fg-105_NoV
fg-106_NoV
fg-107_NoV
D
shocks / interval
time (min)
PBS (n = 3)
S36 (n = 4)
E
distance run (m)
PBS
n = 3
S36
n = 4
F
fatigue time (min)
PBS
n = 3
S36
n = 4

FIG. 2

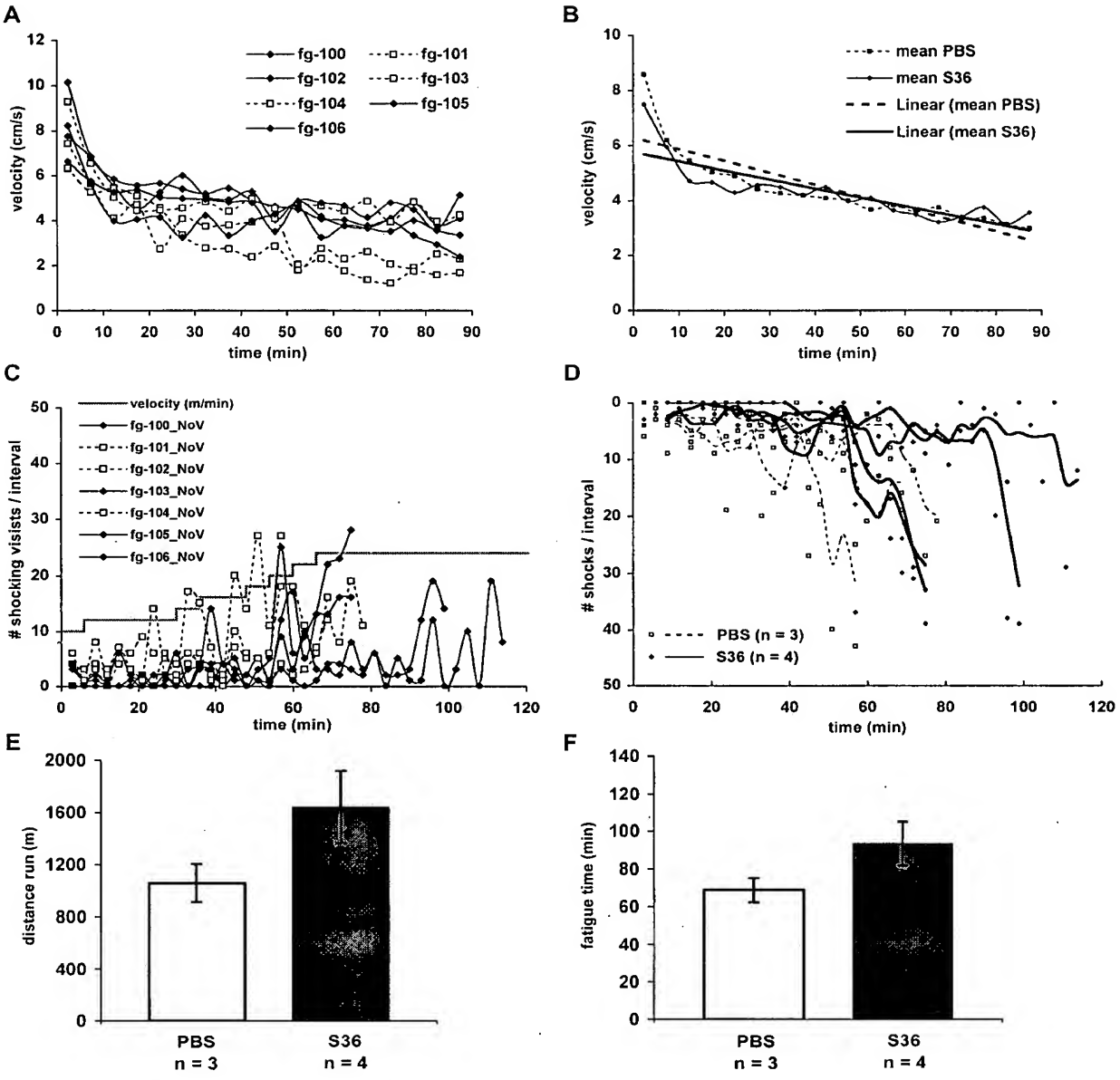

A
velocity (cm/s)
time (min)
fg-100
fg-101
fg-102
fg-103
fg-104
fg-105
fg-106
B
velocity (cm/s)
time (min)
mean PBS
mean S36
Linear (mean PBS)
Linear (mean S36)
C
shocking visists / interval
time (min)
velocity (m/min)
fg-100_NoV
fg-101_NoV
fg-102_NoV
fg-103_NoV
fg-104_NoV
fg-105_NoV
fg-106_NoV
D
shocks / interval
time (min)
PBS (n = 3)
S36 (n = 4)
E
distance run (m)
PBS
n = 3
S36
n = 4
F
fatigue time (min)
PBS
n = 3
S36
n = 4

FIG. 3

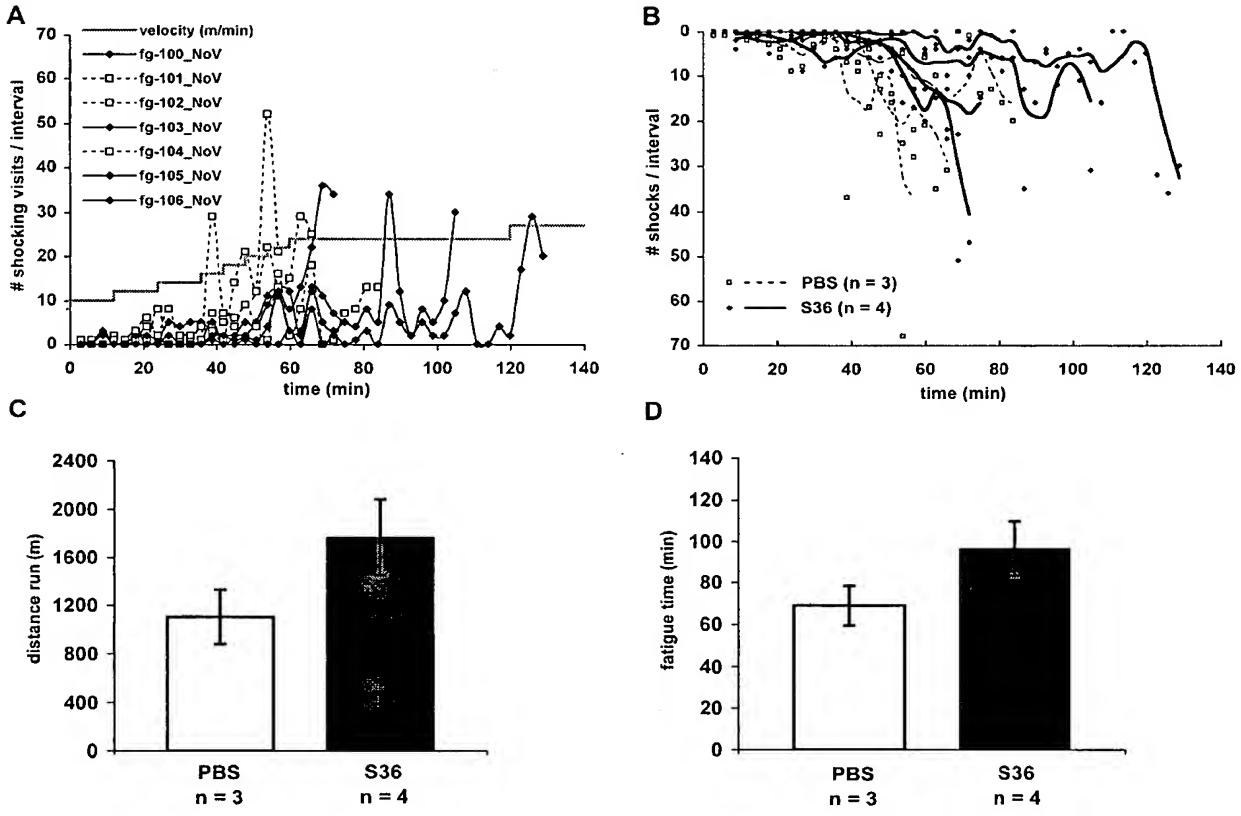

A
velocity (m/min)
fg-100_NoV
fg-101_NoV
fg-102_NoV
fg-103_NoV
fg-104_NoV
fg-105_NoV
fg-106_NoV
shocking visits / interval
time (min)
B
shocks / interval
PBS (n = 3)
S36 (n = 4)
time (min)
C
distance run (m)
PBS
n = 3
S36
n = 4
D
fatigue time (min)
PBS
n = 3
S36
n = 4

FIG. 4

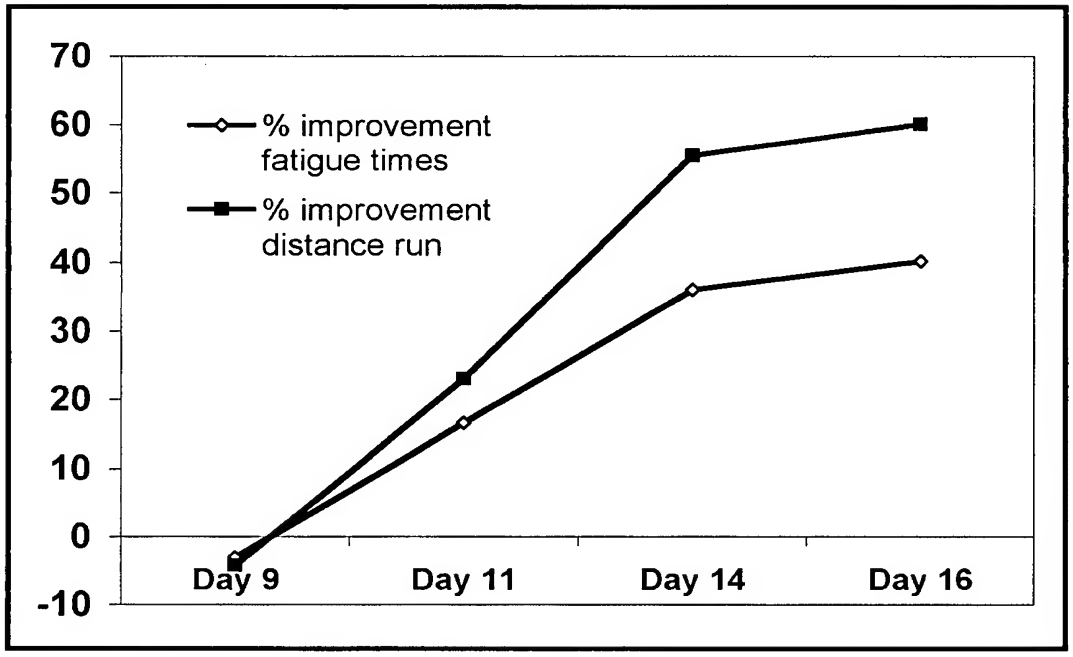

% improvement
fatigue times
% improvement
distance run
Day 9
Day 11
Day 14
Day 16

FIG. 5

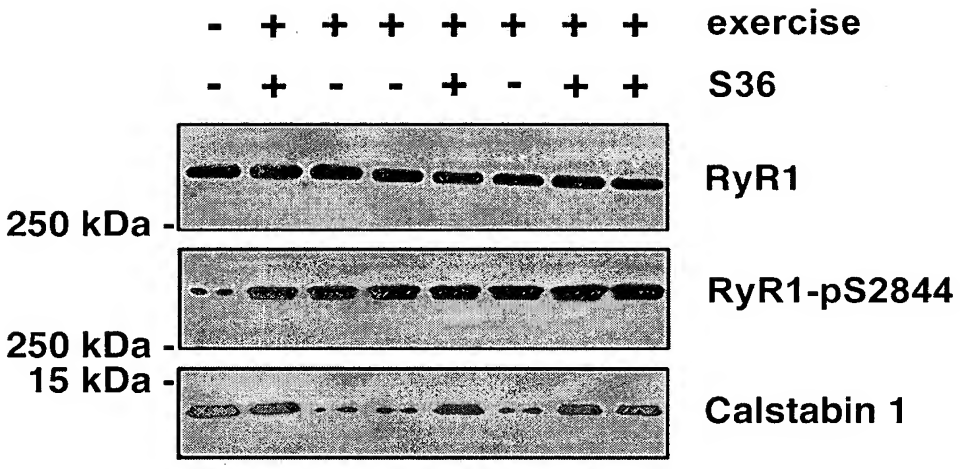

- + + + + + + + exercise
- + - - + - + + S36
250 kDa -
RyR1
RyR1-pS2844
250 kDa -
15 kDa -
Calstabin 1

FIG. 6

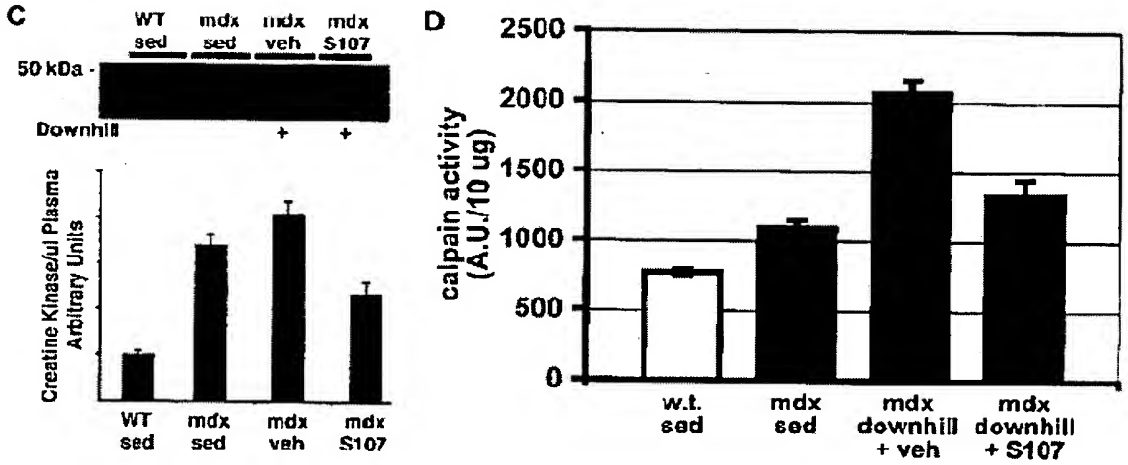
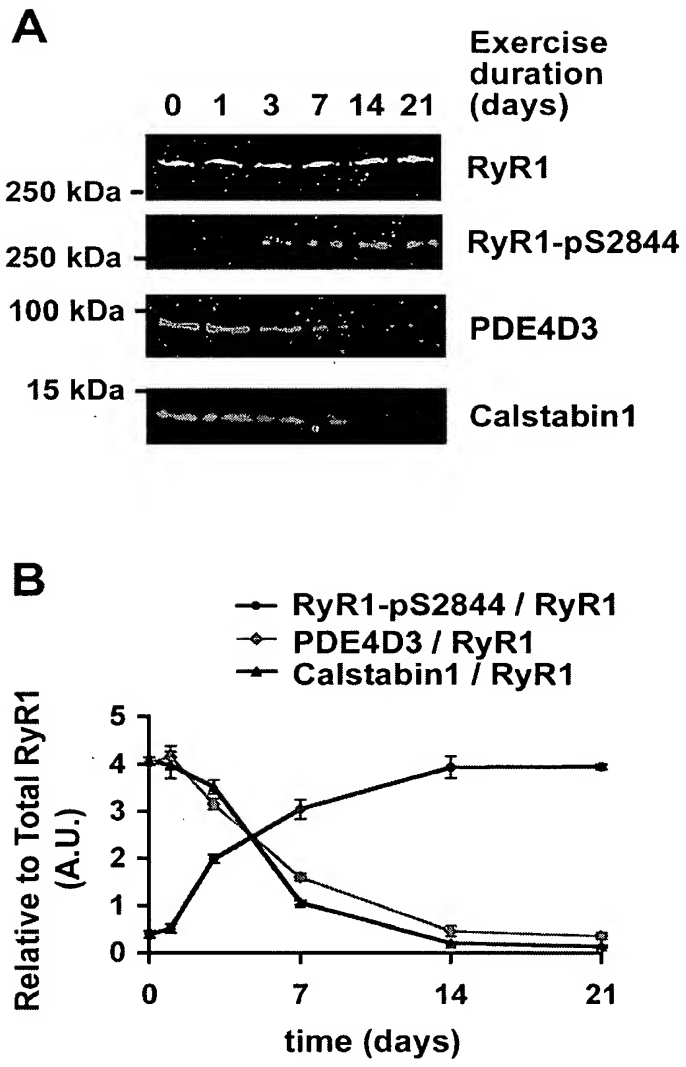
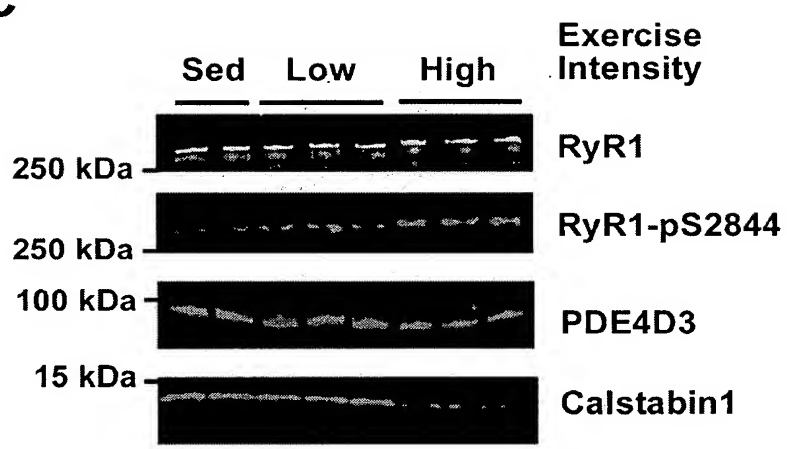

C
WT sed
mdx sed
mdx veh
mdx S107
50 kDa
Downhill
+
+
Creatine Kinase/ul Plasma Arbitrary Units
WT sed
mdx sed
mdx veh
mdx S107
D
2500
2000
1500
1000
500
0
calpain activity (A.U./10 ug)
w.t. sed
mdx sed
mdx downhill + veh
mdx downhill + S107

Fig. 7 C & 7D

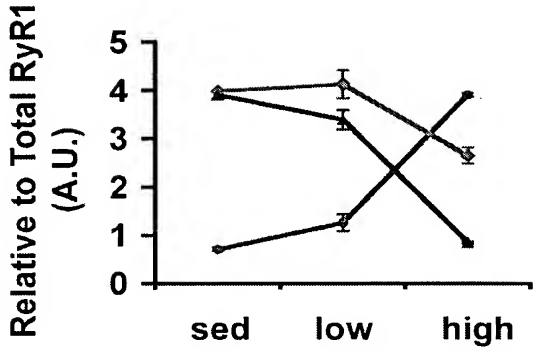

A
Exercise duration (days)
0 1 3 7 14 21
250 kDa
RyR1
250 kDa
RyR1-pS2844
100 kDa
PDE4D3
15 kDa
Calstabin1
B
RyR1-pS2844 / RyR1
PDE4D3 / RyR1
Calstabin1 / RyR1
Relative to Total RyR1 (A.U.)
0
1
2
3
4
5
0
7
14
21
time (days)

FIGS. 8A & 8B

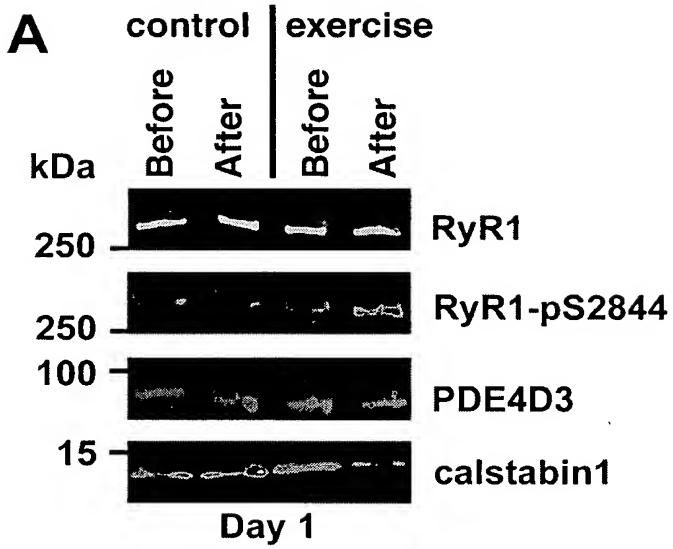
C

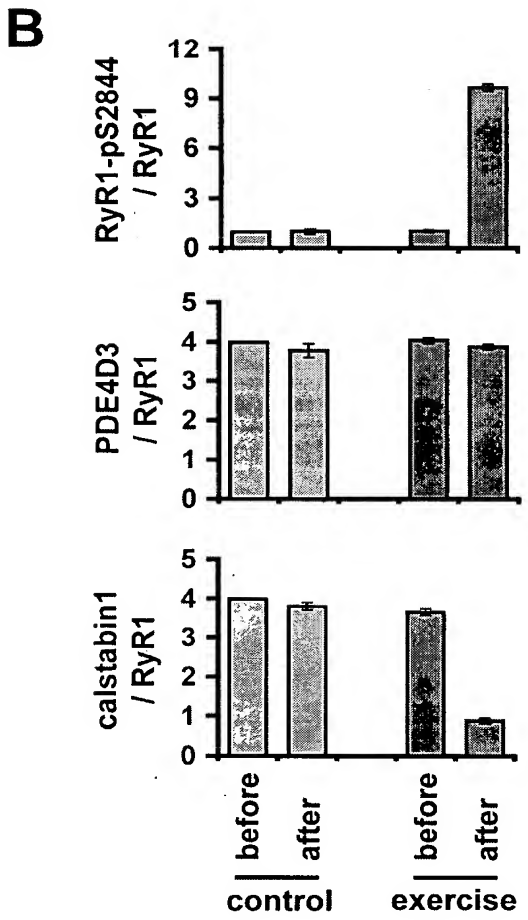

Sed
Low
High
Exercise Intensity
250 kDa
RyR1
250 kDa
RyR1-pS2844
100 kDa
PDE4D3
15 kDa
Calstabin1

D

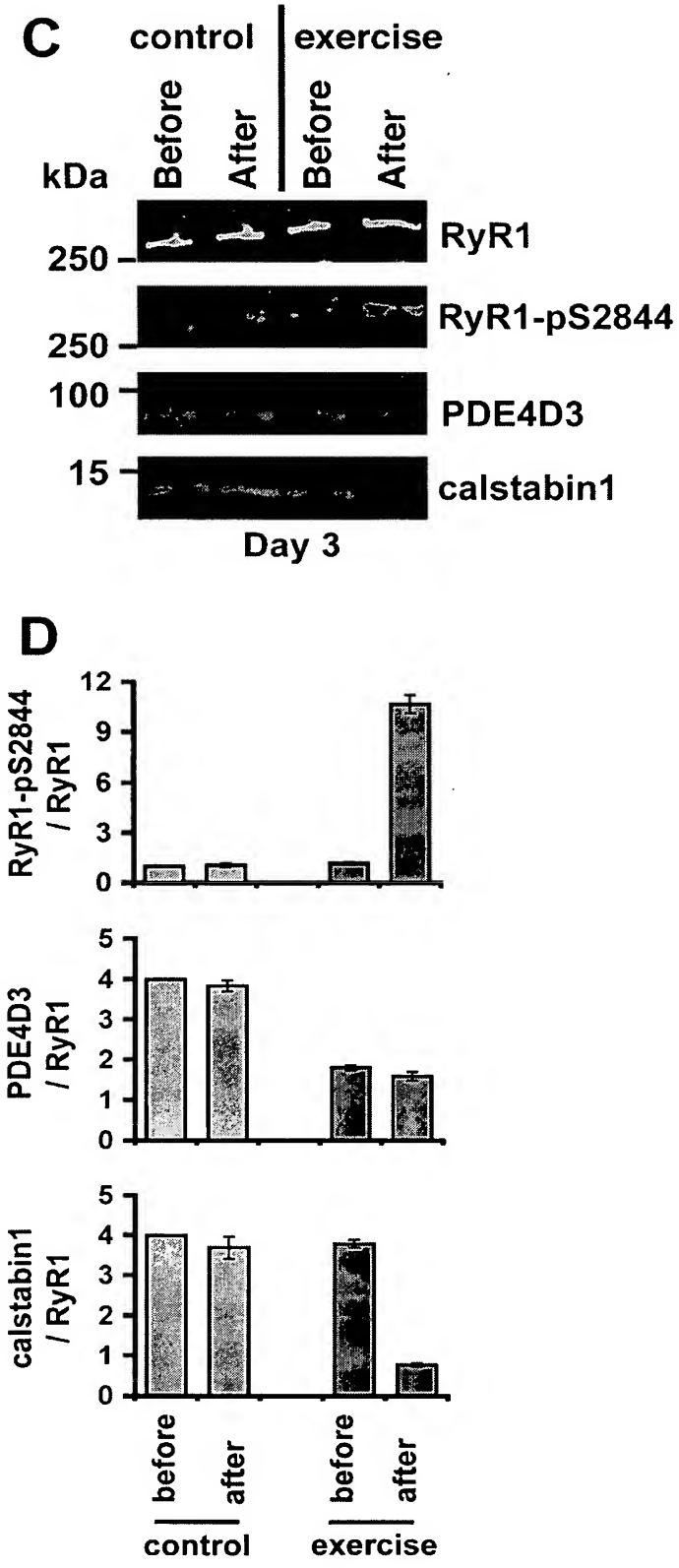

Relative to Total RyR1 (A.U.)
5
4
3
2
1
0
sed
low
high

FIGS. 8C & 8D


A
control
exercise
Before
After
Before
After
kDa
250
RyR1
250
RyR1-pS2844
100
PDE4D3
15
calstabin1
Day 1


B
RyR1-pS2844 / RyR1
12
9
6
3
0
PDE4D3 / RyR1
5
4
3
2
1
0
calstabin1 / RyR1
5
4
3
2
1
0
before
after
before
after
control
exercise

FIGS. 9A & 9B


C
control
exercise
Before
After
Before
After
kDa
250
RyR1
250
RyR1-pS2844
100
PDE4D3
15
calstabin1
Day 3
D
RyR1-pS2844 / RyR1
12
9
6
3
0
PDE4D3 / RyR1
5
4
3
2
1
0
calstabin1 / RyR1
5
4
3
2
1
0
before
after
before
after
control
exercise

FIGS. 9C & 9D

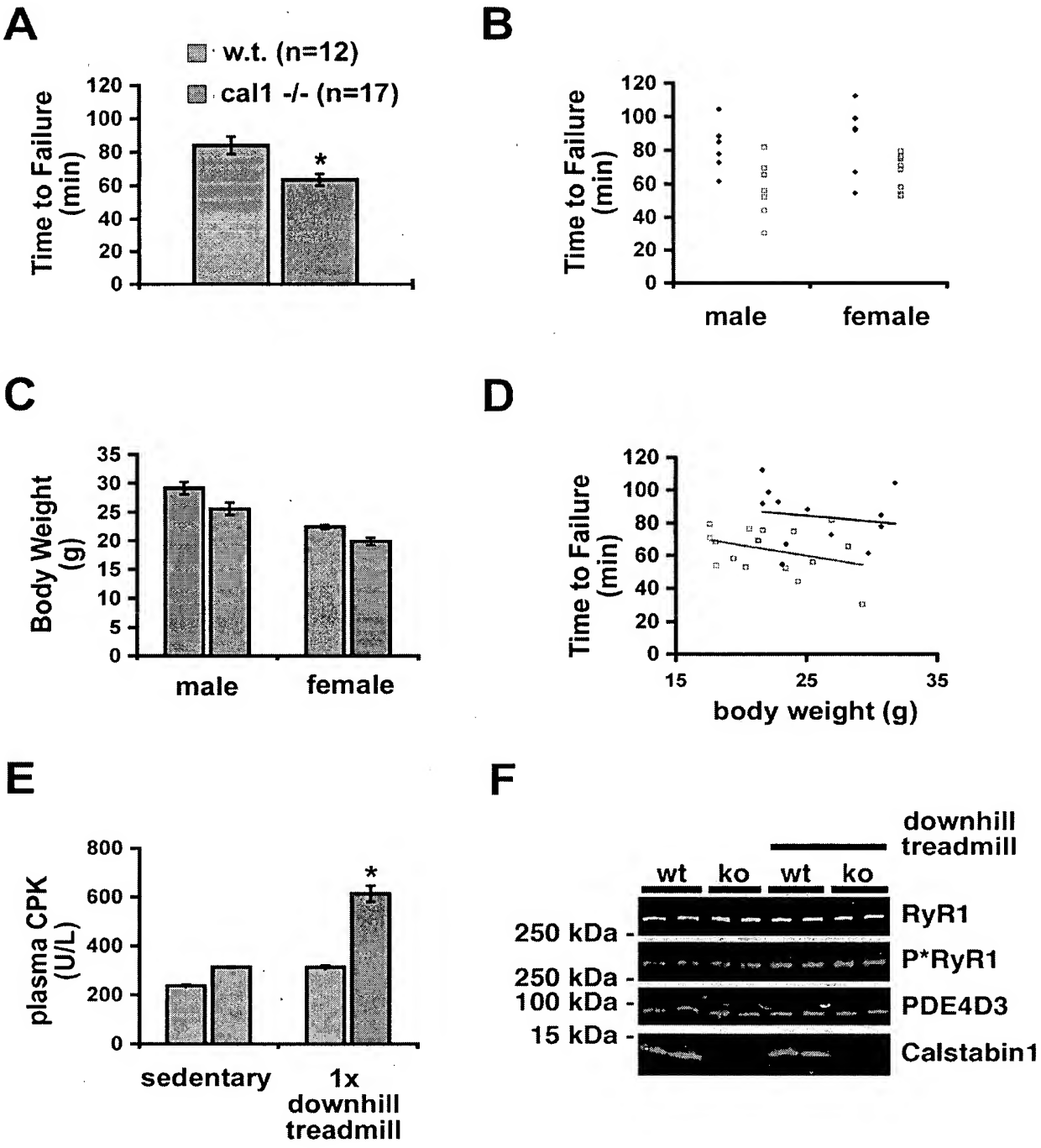
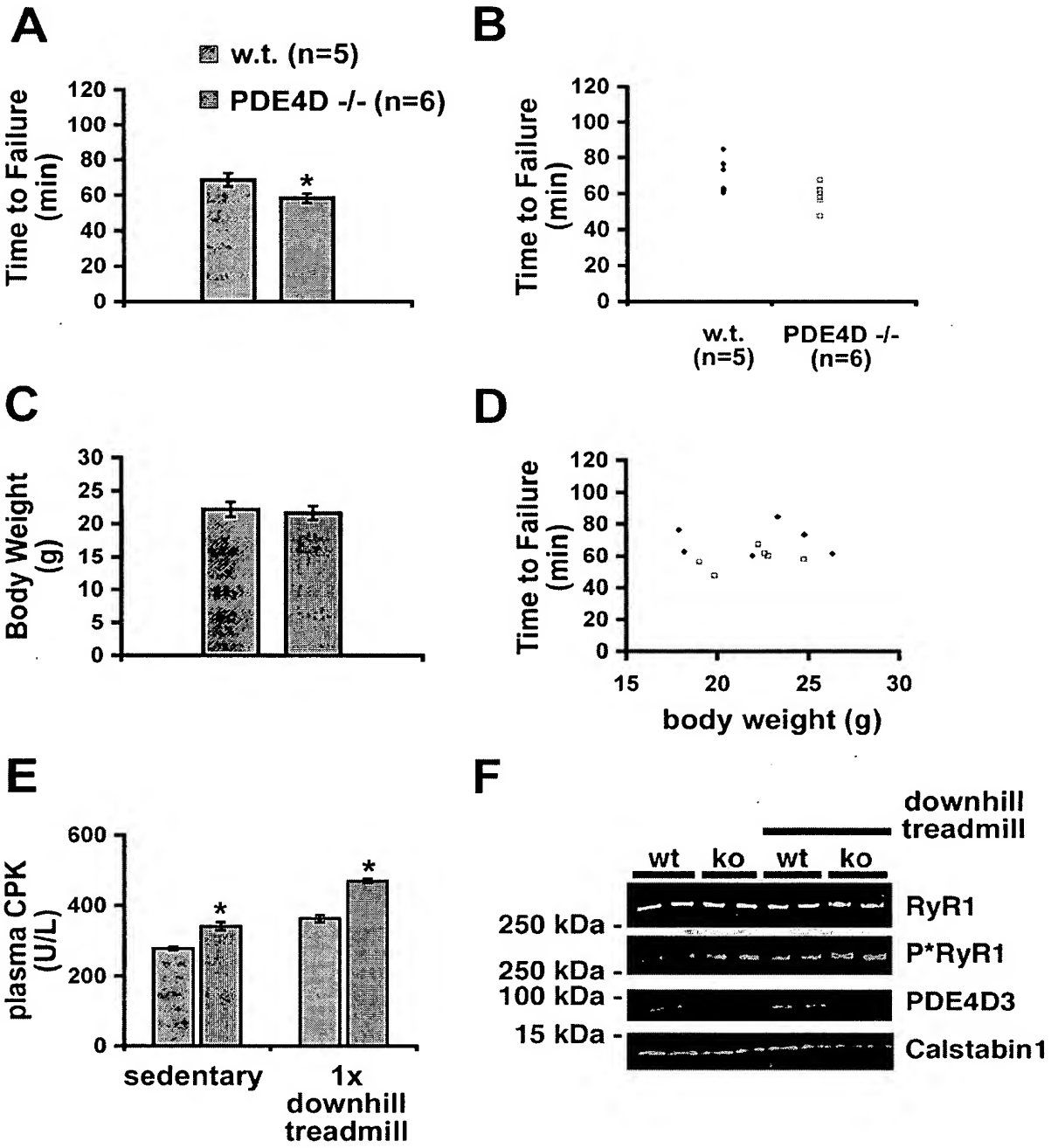
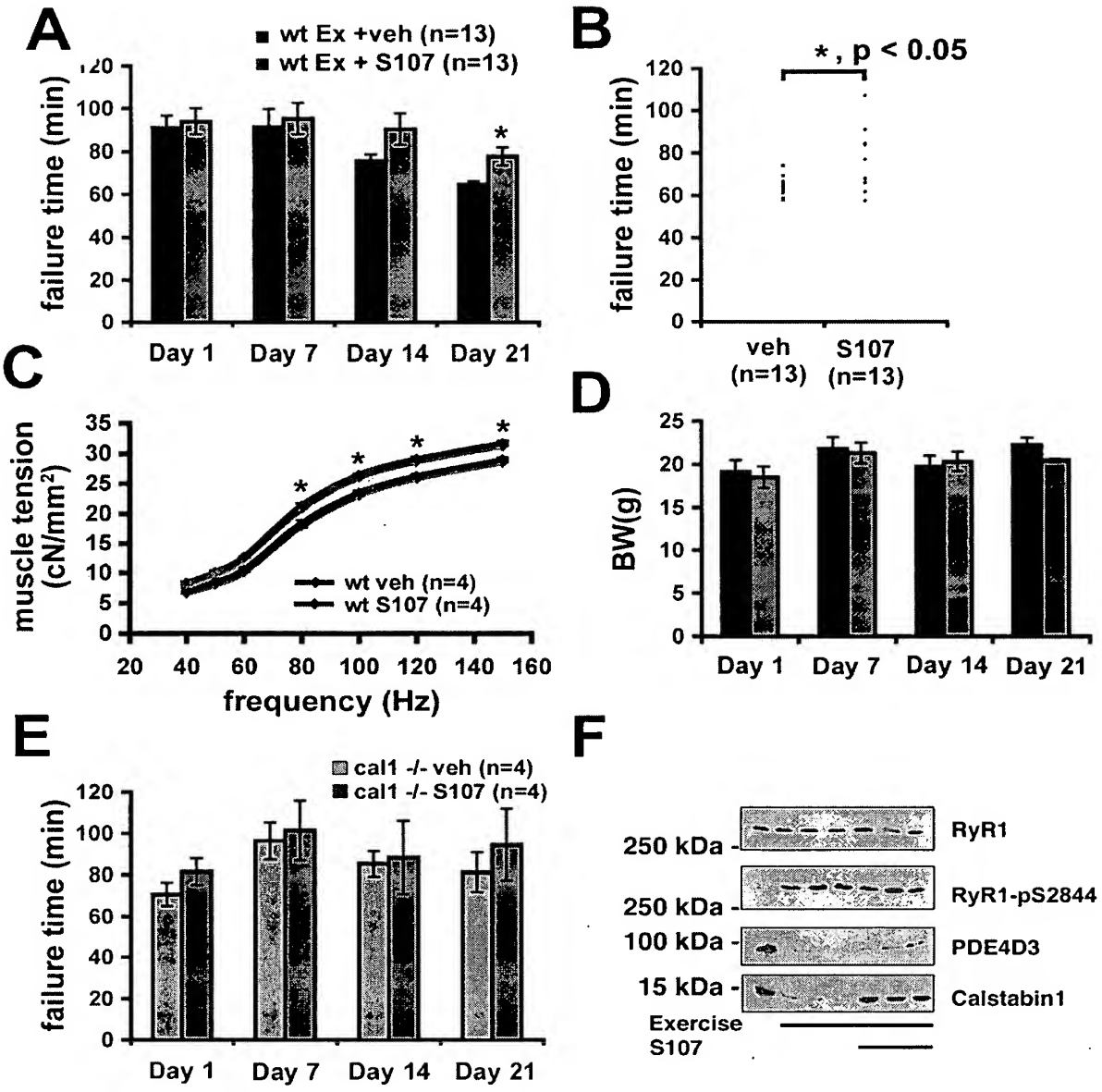

A
w.t. (n=12)
cal1 -/- (n=17)
Time to Failure (min)
120
100
80
60
40
20
0
*
B
Time to Failure (min)
male
female
C
Body Weight (g)
35
30
25
20
15
10
5
0
male
female
D
Time to Failure (min)
body weight (g)
15
25
35
E
plasma CPK (U/L)
800
600
400
200
0
*
sedentary
1x downhill treadmill
F
downhill treadmill
wt
ko
wt
ko
250 kDa
RyR1
250 kDa
P*RyR1
100 kDa
PDE4D3
15 kDa
Calstabin1

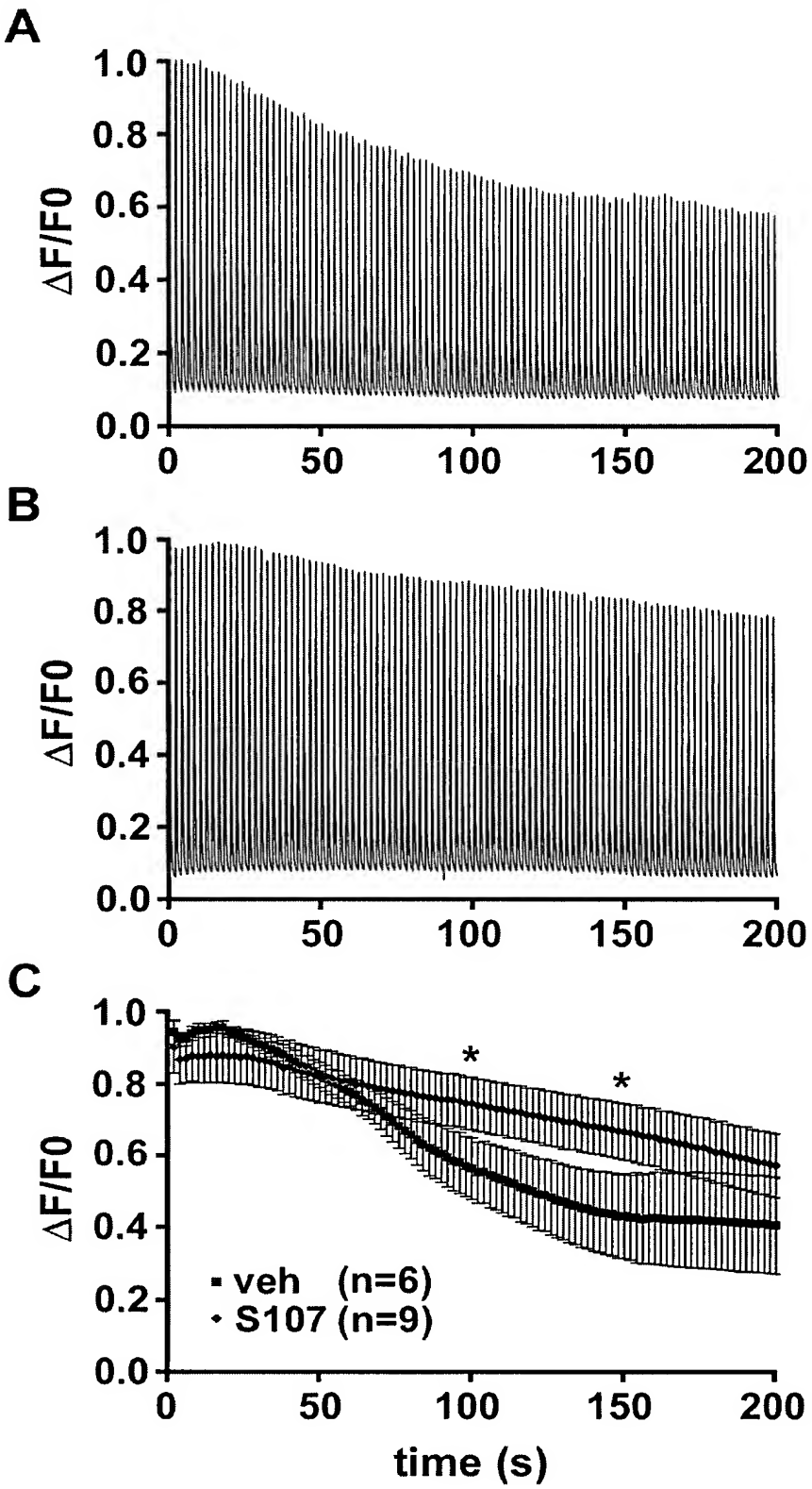
FIG. 10



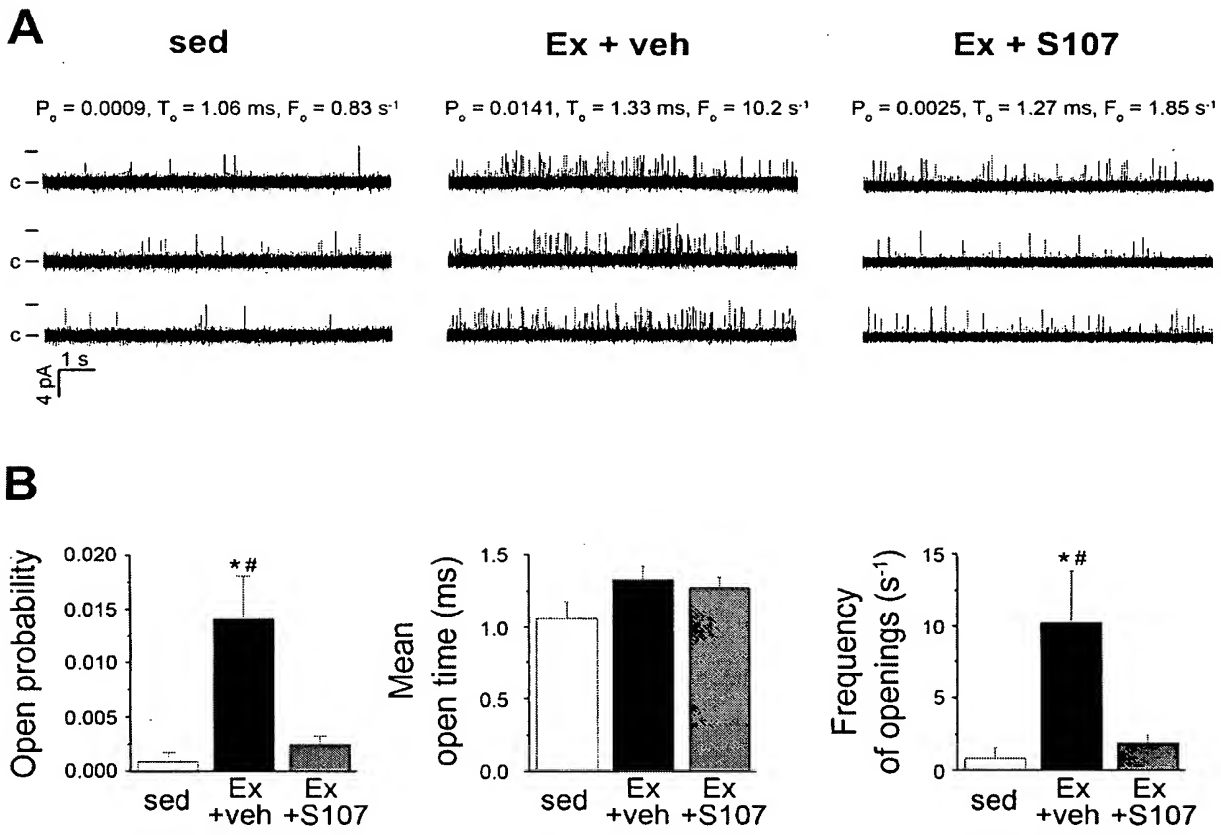
FIGS. 11 A, B, C, D, E, F


A
wt Ex +veh (n=13)
wt Ex + S107 (n=13)
failure time (min)
Day 1
Day 7
Day 14
Day 21
B
*, p < 0.05
failure time (min)
veh (n=13)
S107 (n=13)
C
muscle tension (cN/mm²)
wt veh (n=4)
wt S107 (n=4)
frequency (Hz)
D
BW(g)
Day 1
Day 7
Day 14
Day 21
E
cal1 -/- veh (n=4)
cal1 -/- S107 (n=4)
failure time (min)
Day 1
Day 7
Day 14
Day 21
F
250 kDa
RyR1
250 kDa
RyR1-pS2844
100 kDa
PDE4D3
15 kDa
Calstabin1
Exercise
S107

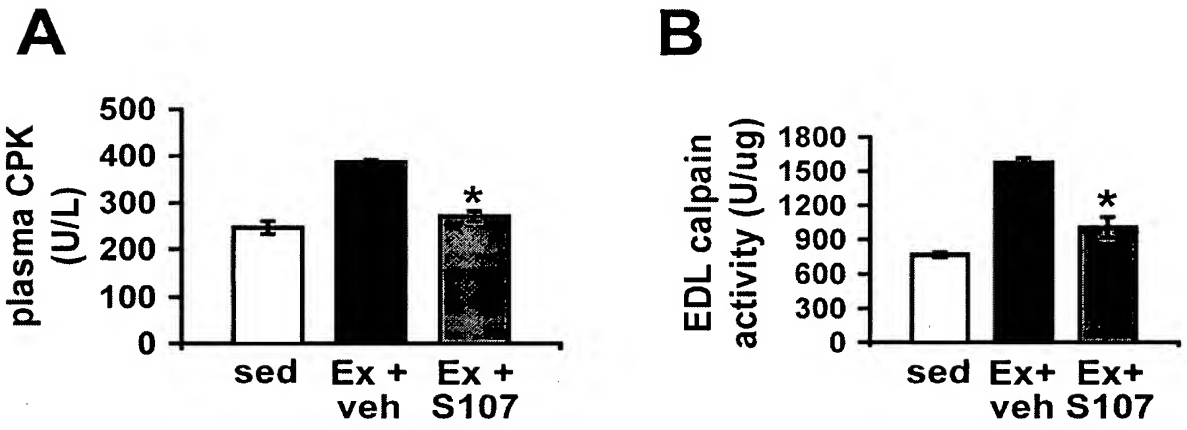
FIGS. 12A-12F



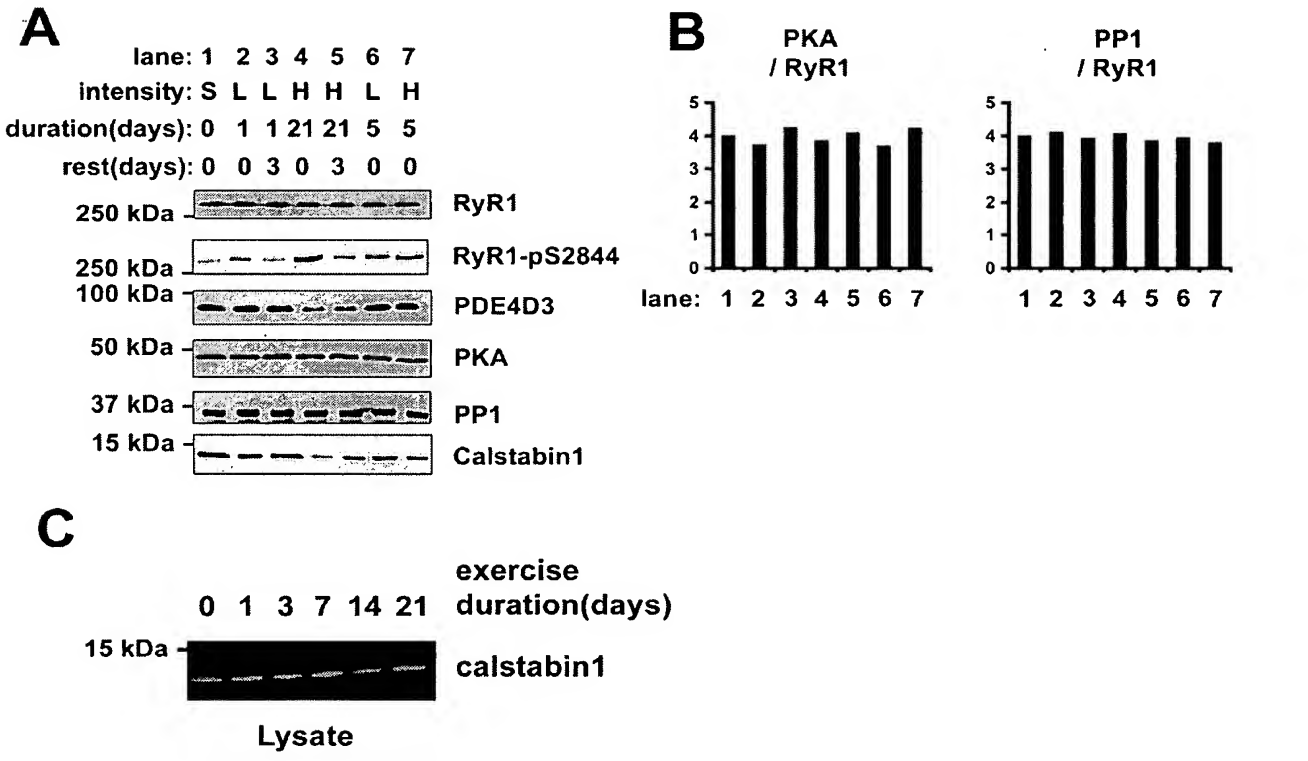
FIGS. 13A-13C



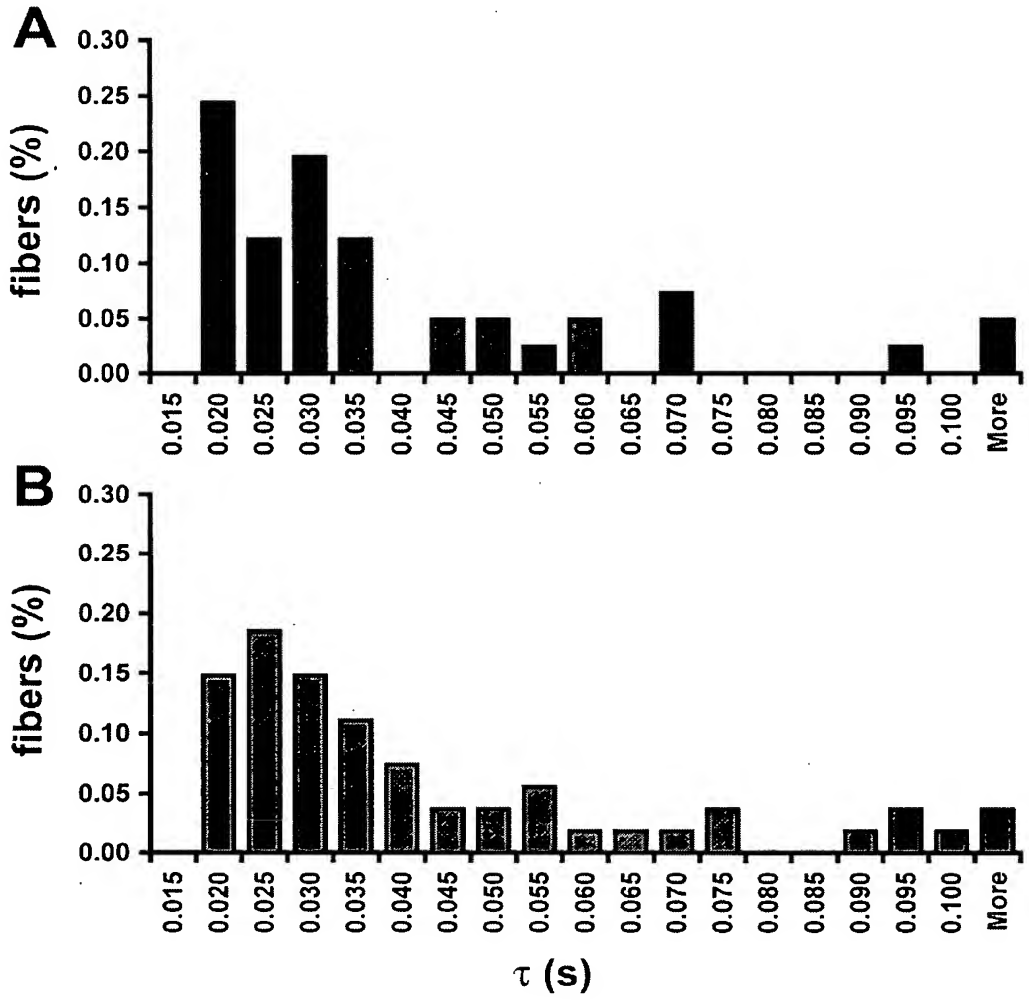
FIGS. 14A-14B



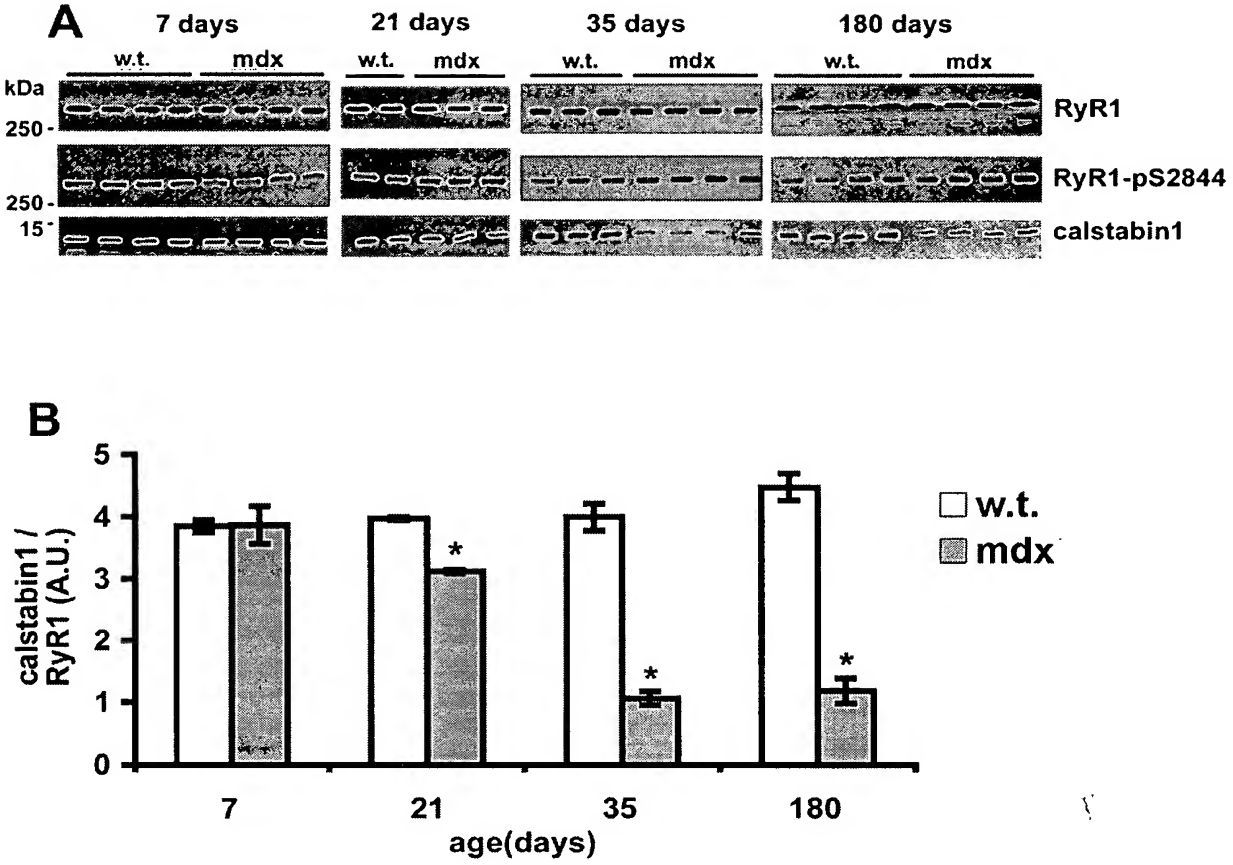
FIGS. 15A-15B


A
lane: 1 2 3 4 5 6 7
intensity: S L L H H L H
duration(days): 0 1 1 21 21 5 5
rest(days): 0 0 3 0 3 0 0
250 kDa
RyR1
250 kDa
RyR1-pS2844
100 kDa
PDE4D3
50 kDa
PKA
37 kDa
PP1
15 kDa
Calstabin1
B
PKA / RyR1
PP1 / RyR1
lane: 1 2 3 4 5 6 7
C
exercise duration(days)
0 1 3 7 14 21
15 kDa
calstabin1
Lysate

FIGS. 16A-16C


A
B
fibers (%)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.015
0.020
0.025
0.030
0.035
0.040
0.045
0.050
0.055
0.060
0.065
0.070
0.075
0.080
0.085
0.090
0.095
0.100
More
τ (s)

FIGS. 17A-17B



FIGS. 18A & 18B

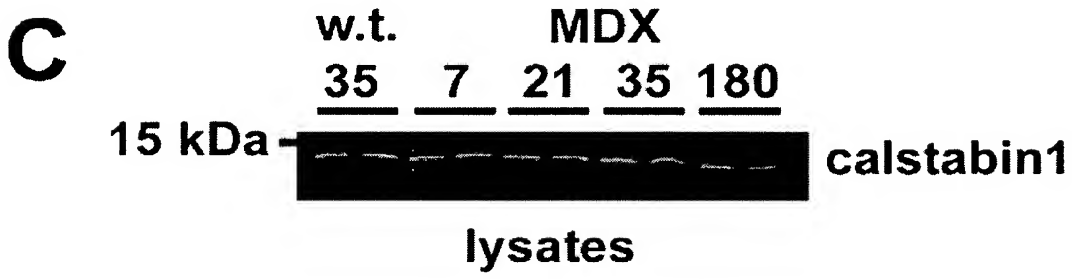
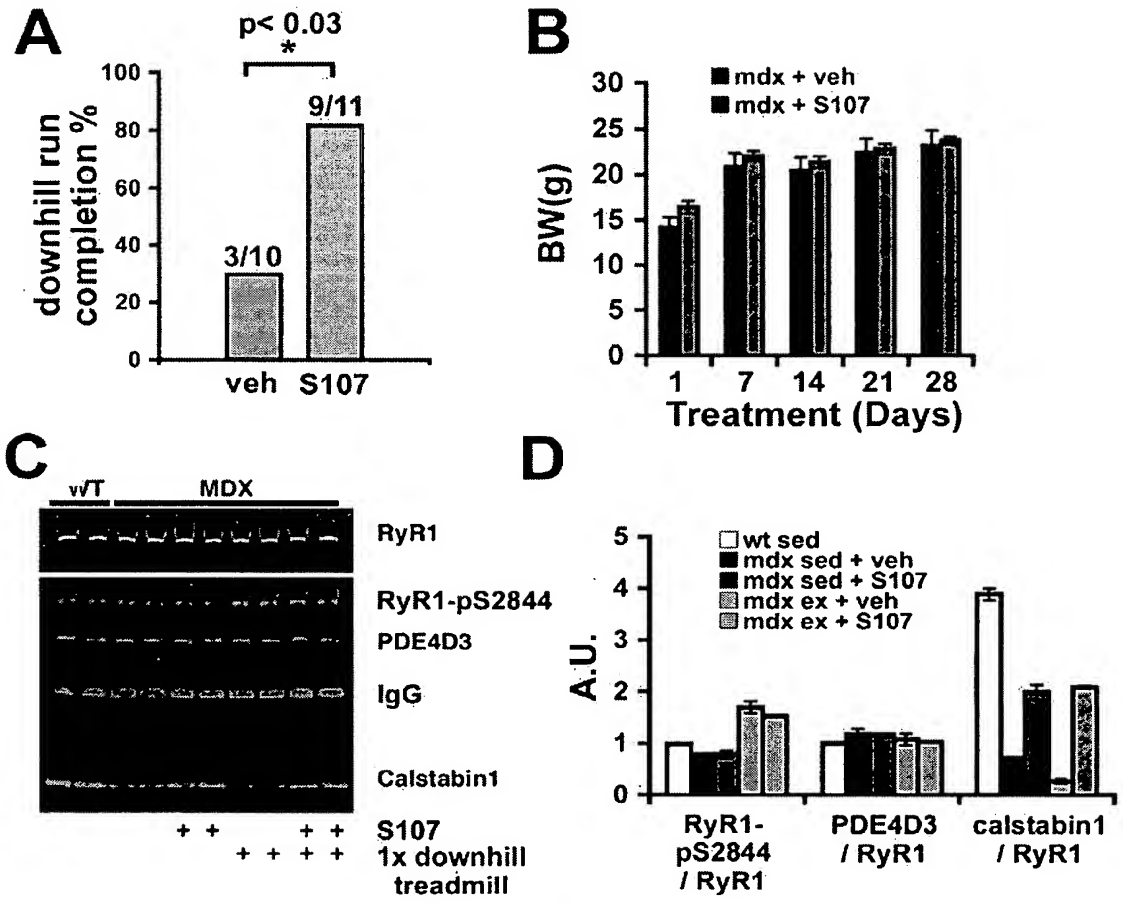


FIG. 18C


A
p< 0.03
*
downhill run completion %
100
80
60
40
20
0
3/10
9/11
veh
S107
B
mdx + veh
mdx + S107
BW(g)
30
25
20
15
10
5
0
1
7
14
21
28
Treatment (Days)
C
WT
MDX
RyR1
RyR1-pS2844
PDE4D3
IgG
Calstabin1
S107
1x downhill treadmill
D
wt sed
mdx sed + veh
mdx sed + S107
mdx ex + veh
mdx ex + S107
A.U.
5
4
3
2
1
0
RyR1-pS2844 / RyR1
PDE4D3 / RyR1
calstabin1 / RyR1
E
plasma CPK (U/L)
1500
1200
900
600
300
0
F
EDL calpain activity (U/ug)
2500
2000
1500
1000
500
0

FIGS. 19A-19F

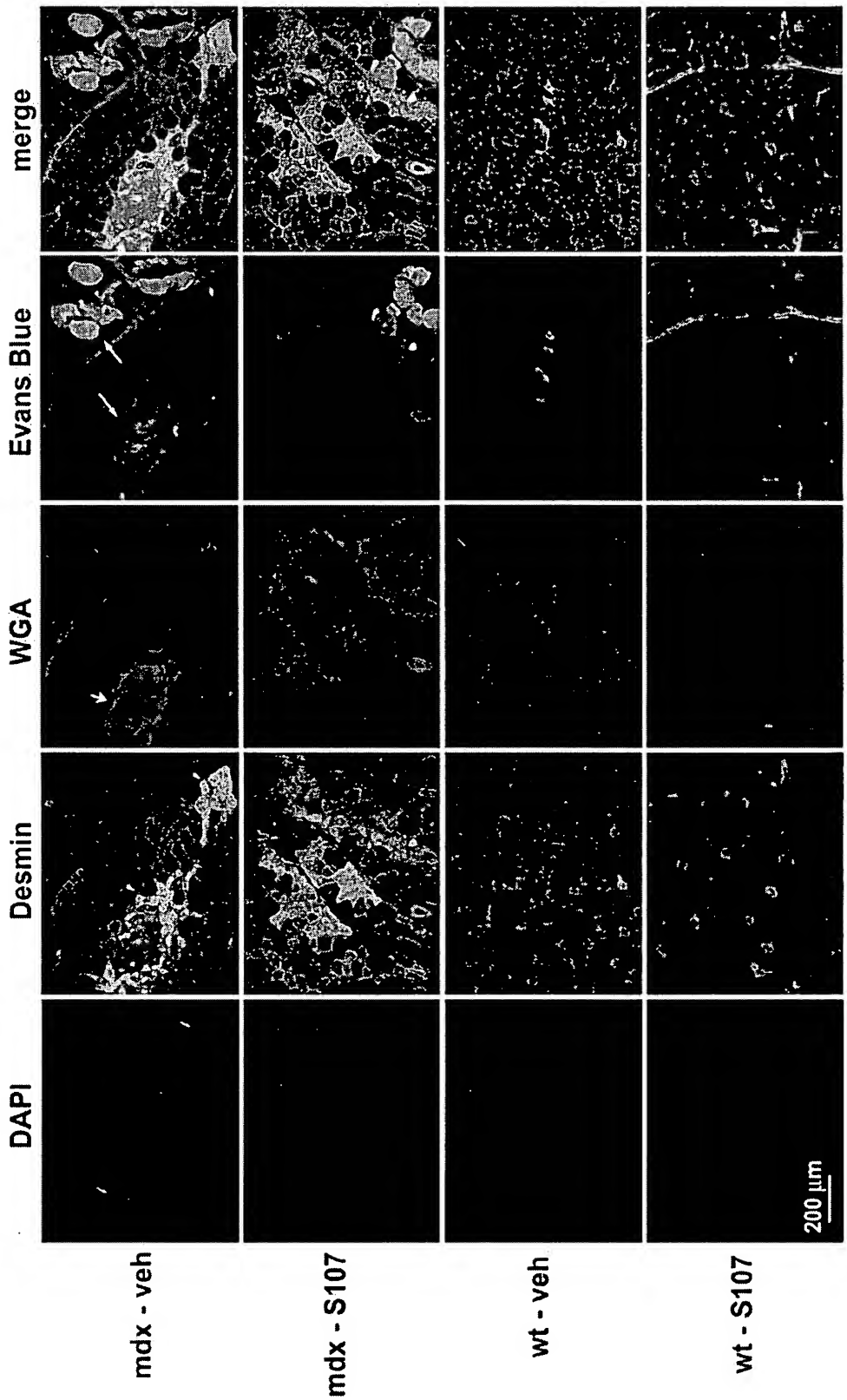

DAPI
Desmin
WGA
Evans Blue
merge
mdx - veh
mdx - S107
wt - veh
wt - S107
200 µm

FIG. 20